COMPARISON OF ANAEROBIC BAFFLED (ABR) AND MIGRATING BLANKET REACTORS (AMBR) IN THE ANAEROBIC TREATABILITY OF NITROORGANIC COMPOUNDS

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by
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İZMİR

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We have read the thesis entitled **"COMPARISON OF ANAEROBIC BAFFLED (ABR) AND MIGRATING BLANKET REACTORS (AMBR) IN THE ANAEROBIC TREATABILITY OF NITROORGANIC COMPOUNDS"** completed by **Özlem SELÇUK KUŞÇU** under supervision of **Prof. Dr. Delia Teresa SPONZA** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Philosophy.

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Özlem SELÇUK KUŞÇU

COMPARISON OF ANAEROBIC BAFFLED (ABR) AND MIGRATING BLANKET REACTORS (AMBR) IN THE ANAEROBIC TREATABILITY OF NITROORGANIC COMPOUNDS

ABSTRACT

In the framework of this Ph. D. thesis, the treatability of p-nitrophenol (p-NP) and nitrobenzene (NB), which are toxic, inhibitory and biorefractory nitroorganic compounds were compared in an sequential Anaerobic Migrating Blanked Reactor (AMBR)/aerobic Continuously Stirred Tank Reactor (CSTR) and sequential Anaerobic Baffled Reactor (ABR)/aerobic Continuously Stirred Tank Reactor (CSTR) reactor systems at increasing p-NP and NB loading rates and six different HRTs. COD, p-NP and NB removal efficiencies, total gas, methane gas productions, methane percentage, TVFA, Bic.Alk., TVFA/Bic.Alk. ratios were investigated separately in AMBR and ABR reactors at increasing p-NP and NB loading rates and decreasing HRTs. Furthermore, appropriate substrate and biogas kinetic models were determined for AMBR and ABR reactors treating p-NP.

High COD, p-NP and NB removal efficiencies were obtained at HRTs varied between 10.38 days and 3.5 days in AMBR and ABR reactors. Methane gas percentages were high between these HRTs. pH, TVFA, Bic.Alk. and TVFA/Bic.Alk. ratios were found between optimum values in AMBR and ABR reactors through continuous operation. Toxic p-NP and NB transformed to less toxic intermediate products in anaerobic/aerobic sequential reactor system. In this study, p-NP transformed to p-aminophenol under anaerobic conditions. NB transformed to aniline under anaerobic conditions, then aniline biodegraded to catechol under aerobic conditions. The appropriate substrate removal and biogas production kinetic models were found as Modified Stover Kincannon model for AMBR and ABR reactors treating p-NP. The results of this study showed that these reactors can be used effectively to treat the toxic wastewaters containing p-NP and NB.

Keywords: Anaerobic migrating blanked reactor (AMBR), anaerobic baffled reactor (ABR), para-nitrophenol (p-NP), nitrobenzene (NB), anaerobic treatment, anaerobic/aerobic treatment, p-AP, aniline, toxicity.

NİTROORGANİK BİLEŞİKLERİN ANAEROBİK ARITILABİLİRLİĞİNDE ANAEROBİK PERDELİ (APR) VE HAREKETLİ YATAK REAKTÖR (AHYR)'ÜN KARŞILAŞTIRILMASI

ÖZ

Bu tez kapsamında toksik, engelleyici ve çevrede zor parçalanabilir nitroorganik bileşiklerden olan p-nitrofenol (p-NF) ve nitrobenzenin (NB) arıtılabilirliği, ardışık Anaerobik Hareketli Yatak Reaktör (AHYR)/ aerobik Sürekli Karıştırmalı Tank Reaktör (SKTR) ve ardışık Anaerobik Perdeli Reaktör (APR)/ aerobik Sürekli Karıştırmalı Tank Reaktör (SKTR) sistemlerinde, artan p-NF ve NB yükleme hızlarında ve altı farklı hidrolik bekleme sürelerinde (HBS) karşılaştırılmıştır. KOİ, p-NF ve NB uzaklaştırma verimleri, toplam gaz, metan gaz üretimleri, metan yüzdesi, TUYA, Bik.Alk. ve TUYA/Bik.Alk. oranları değişimleri artan p-NF ve NB yükleme hızlarında ve azalan HBS' lerde AHYR ve APR'de ayrı ayrı incelenmiştir. Ayrıca, p-NF arıtan AHYR ve APR reaktörler için uygun substrat giderim ve biyogaz üretim kinetik modellerine karar verilmiştir.

AHYR ve APR reaktörlerde yüksek KOİ, p-NF ve NB uzaklaştırma verimleri 10.38 ila 3.5 gün arasında değişen HBS'lerde elde edilmiştir. Metan gaz yüzdesi, bu HBS' ler arasında yüksek bulunmuştur. AHYR ve APR reaktörlerinde, pH, TUYA, Bik.Alk. ve TUYA/Bik.Alk. oranları sürekli işletim süresince optimum değerler arasında kalmıştır. Toksik p-NF ve NB, ardışık anaerobik/aerobik reaktör sisteminde daha az toksik ara ürünlere dönüşmüştür. Bu çalışmada, p-NF anaerobik şartlar altında p-aminofenole dönüşmüştür. NB ise anaerobik şartlar altında aniline, aniline ise aerobik şartlar altında kateşole dönüşmüştür. AHYR ve APR reaktörler için uygun substrat giderim ve biyogaz üretim kinetik modeli, modifiye edilmiş Stover Kincannon model olarak bulunmuştur. Bu çalışma sonuçları, bu reaktörlerin, p-NF ve NB içeren toksik atıksularının arıtımımda etkili bir şekilde kullanılabileceğini göstermektedir.

Anahtar kelimeler: Anaerobik hareketli yatak reaktör (AHYR), anaerobik perdeli reaktör (APR), para-nitrofenol (p-NF), nitrobenzen (NB), anaerobik arıtım, anaerobik/aerobik arıtım, p-AF, anilin, toksisite.

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CHAPTER ONE
INTRODUCTION

1.1 Introduction

Nitroaromatic compounds such as nitrophenol and nitrobenzene are widely used as raw materials or intermediates in the manufacture of explosives, pharmaceuticals, pesticides, pigments, plastics, dyes, wood preservatives, leather and rubber chemicals (Bhatti, Toda, & Furukawa, 2002; Karim & Gupta, 2003; Uberoi & Bhattacharya, 1997; Ye, Singh, & Owen, 2004). Nitroorganic compounds, their byproducts and metabolites can be highly toxic, mutagenic and carcinogenic for the environment and human health (Ye, Singh, & Owen, 2004). These compounds are generally recalcitrant to biological treatment and remain in the environment. Nitrophenol and nitrobenzene were listed by the U.S. Environmental Protection Agency (EPA)’s as “Priority Pollutants” (EPA, 1994).

Nitroorganic compounds are generally considered to be highly resistant to microbial degradation. The purification of wastewaters contaminated with these pollutants is very difficult since they are resistant to the conventional treatment techniques (Paola, Palmisano, Pantaleo, & Savinov, 2003). Combined anaerobic/aerobic processes are a viable alternative for the treatment of nitroorganic compounds that are difficult to treat by traditional processes (Speece, 1996). Generally, aerobic polishing step is needed after the anaerobic system (pretreatment) to meet the effluent quality standards (Angenent & Sung, 2001).

The anaerobic baffled reactor (ABR) and anaerobic migrating blanked reactor (AMBR) are high rate anaerobic reactors offering two-phase separation with a single vessel. The literature survey shows that there is a lack on the anaerobic treatment of p-nitrophenol and nitrobenzene by ABR and AMBR. In other words, no study was found in the literature for the AMBR and ABR reactor treating the wastewaters containing p-nitrophenol (p-NP) and nitrobenzene (NB).

1.2 The Objective and Scope of the Study

The general objective of this study was to evaluate of the performance of the anaerobic migrating blanked reactor (AMBR) and anaerobic baffled reactor (ABR) and to investigate of the effect of their compartments on the treatment efficiency during various hydraulic retention time (HRT) and organic loading rates using synthetic wastewater containing p-NP and NB, separately. The specific objectives of this study are as follows:

1. To determine the inhibition concentration of p-NP and NB which caused 50% decrease in the methanogenic activity (IC_{50}) in batch serum bottles. The batch studies gives information about the p-NP and NB doses will be used in the AMBR and ABR reactor through continuous operation.

2. To determine the para-nitropfenol (p-NP), nitrobenzene (NB) and dissolved chemical oxygen demand (COD) removal efficiencies, total gas, methane gas productions, methane percentages in AMBR and ABR reactors at increasing p-NP and NB loading rates under constant hydraulic retention times (HRT). Furthermore to determine the effect of compartments, located in the reactors, on the total reactor performances based on p-NP, NB, COD, pH, total volatile fatty acid (TVFA), bicarbonate alkalinity (Bic.Alk.) and TVFA/Bic.Alk. ratios at increasing p-NP and NB loading rates under constant HRTs.

3. To determine total removal efficiency in sequential anaerobic AMBR/ completely stirred tank rector (CSTR) and sequential anaerobic ABR/ completely stirred tank rector (CSTR) systems at increasing p-NP and NB loading rates under constant HRTs.

4. To determine p-NP, NB and COD removal efficiencies, total gas, methane gas productions, methane percentages in AMBR and ABR reactors at decreasing HRTs under constant p-NP and NB concentrations, separately. Furthermore to determine the effect of compartments, located in the reactors, on the total reactor performances based on p-NP, NB, COD, pH, total volatile fatty acid,

bicarbonate alkalinity (Bic.Alk.) and TVFA/Bic.Alk. ratios at decreasing HRTs under constant p-NP and NB concentrations.

5. To determine total removal efficiency in sequential anaerobic AMBR/ completely stirred tank rector (CSTR) and anaerobic ABR/ completely stirred tank rector (CSTR) systems at decreasing hydraulic retention times (HRTs) under constant p-NP and NB concentration.

6. To determine the toxicity effect of p-NP and NB through anaerobic/aerobic degradation in AMBR/CSTR and ABR/CSTR reactor systems operated at increasing p-NP and NB loading rates and different HRTs.

7. To determine the biodegradation products of p-NP and NB through continuous operation of anaerobic/aerobic reactor systems.

8. To determine the substrate, p-NP and NB removal kinetics through continuous operation of anaerobic AMBR and ABR reactors. Furthermore to determine a suitable kinetic model for gas productions and gas quality at different HRTs.

In the first step of this study, the toxic effect of p-NP and NB on methane *Archaea* was investigated using anaerobic toxicity (ATA) test under batch conditions in the beginning of the study in order to determine the IC_{50} (The p-NP and NB concentrations which caused 50% decrease in the methanogenic activity) values of the p-NP and NB.

In the second step of this study COD and p-NP treatabilities were studied in a sequential anaerobic AMBR/ aerobic completely stirred tank reactor (CSTR) reactor system and a sequential anaerobic ABR/aerobic completely stirred tank reactor (CSTR) reactor system at increasing p-NP concentrations under constant flow rates. In this study, the COD, p-NP removal efficiencies, total and methane gas productions, methane gas percentage were investigated at increasing p-NP concentrations under constant flow rates. Furthermore the effects of compartments on the total reactor performances were determined with measuring p-NP, COD, pH, total volatile fatty acid, bicarbonate alkalinity (Bic.Alk.) and TVFA/Bic.Alk. ratios at increasing p-NP concentrations and constant HRTs.

In the third step of this study COD and p-NP treatabilities were studied in a sequential anaerobic AMBR/ aerobic CSTR reactor system and a sequential anaerobic ABR/aerobic CSTR reactor system at different HRTs under constant p-NP concentrations. In this study, the COD, p-NP removal efficiencies, total and methane gas productions, methane gas percentage were investigated at increasing flow rates. Furthermore the effects of compartments on the total reactor performances was determined with measuring p-NP, COD, pH, total volatile fatty acid, bicarbonate alkalinity (Bic.Alk.) and TVFA/Bic.Alk. ratios at decreasing HRTs and constant p-NP concentration. The toxic effect of synthetic wastewater containing p-NP was investigated through anaerobic/aerobic degradation at decreasing HRTs using lumistox and daphnia magna tests.

In the fourth step of this study COD and NB treatabilities were studied in a sequential anaerobic AMBR/ aerobic CSTR reactor system and a sequential anaerobic ABR/aerobic CSTR reactor system at increasing NB concentrations under constant flow rates. In this study, the COD, NB removal efficiencies, total and methane gas productions, methane gas percentage were investigated at increasing NB concentrations under constant flow rates. Furthermore the effects of compartments on the total reactor performances were determined with measuring NB, COD, pH, total volatile fatty acid, bicarbonate alkalinity (Bic.Alk.) and TVFA/Bic.Alk. ratios at increasing NB concentrations.

In the fifty step of this study COD and NB treatabilities were studied in a sequential anaerobic AMBR/aerobic CSTR reactor system and a sequential anaerobic ABR/aerobic CSTR reactor system at different HRTs under constant NB concentrations. In this study, the COD, NB removal efficiencies, total and methane gas productions, methane gas percentage were investigated at increasing flow rates. Furthermore the effects of compartments on the total reactor performances was determined with measuring NB, COD, pH, total volatile fatty acid, bicarbonate alkalinity (Bic.Alk.) and TVFA/Bic.Alk. ratios at different HRTs and constant NB concentration. The toxic effect of synthetic wastewater containing NB was investigated through anaerobic/aerobic degradation at decreasing HRTs using lumistox test.

In the sixty step of this study, different kinetic models such as Monod, Contois, Stover-Kincannon, Grau-second order, Zero order, First order and Second order to the experimental date obtained from the continuous operation of AMBR and ABR reactors were applied to determine the suitable subsrate removal kinetic and relevant kinetic constants under different HRTs. Furthermore different gas production models such as Modified Stover-Kincannon, Chen and Hasminoto and Van der Meer and Heertjes kinetics were used in order to obtain the total and methane gas productions and relevant kinetic constants.

1.3 The Novelties of the Study

The novelties of the study can be summarized as follows:

1. The compartmentalisation structure of the AMBR and ABR reactors increase the treatment efficiencies of anaerobic reactors. The first compartments play as acidogen phase while the subsequent compartments play as methanogen phases to treat the COD, TVFA, p-NP, NB and intermediate products in the both reactors.

2. The anaerobic substrate removal kinetics and biogas kinetics were investigated in AMBR and ABR reactors.

3. The addition of aerobic (CSTR) reactor on the effluent of the AMBR and ABR reactors improve the removal efficiencies by removing the remaining COD, p-NP, NB and inter metabolites entering from the anaerobic reactor resulting in a sequential anaerobic/aerobic reactor system.

CHAPTER TWO
LITERATURE REVIEW

2.1 Nitroaromatic Compounds

The use of nitroorganic compounds as explosives, pesticides, dyes and intermediates in many chemicals syntheses caused their entrance in the environment. Since organic nitro compounds of natural origin are very rare, nitroaromatic compounds nowadays found in the aqueous systems, terretrial system and the atmosphere were formed by human activities (Bosma, Harms, & Zehnder, 2001).

Nitroaromatic compounds may be toxic and/or mutagenic to microorganisms, plant, animals and humans. 2,4,6-Trinitrotoluene (TNT), one of the most abundantly produced nitro compunds, has been shown to cause anemia in humans **(**Bosma, Harms, & Zehnder, 2001). The toxicity of these compounds and their recalcitrant nature can prove to be problematic for their effective biological treatment (Razo-Flores, et al., 1997). In spite of their recalcitrant, microorganisms have developed enzymatic mechanisms to degrade nitro compounds.

The recalcitrance of a nitroaromatic compound is caused by the fact that the nitro groups reduce the electron density of the aromatic ring, so called ‘’ electron-withdrawing effect’’ resulting in impediment of electrophilic attack. It is generally accepted that the higher number of nitro groups on an aromatic ring, indicates the high stability of the aromatic rings against microbial attack (Bang, 1997).

The specific functional nitro-group plays a key role in their conversion mechanisms. Nitoaromatics protect from initial attack by oxygenases due to electron-withdrawing effect. However is favorable for reductive attack to anaerobic bacteria. On the other hand, these compounds are readily reduced via anaerobes to nitroamine ($-NH_2$). Therefore the nitroaromatics are either persist or became amine and products in the anaerobic environment (Ye, Singh, & Owen, 2004).

2.1.1 para-Nitrophenol (p-NP)

2.1.1.1 The Physical and Chemical Characteristics of the p-NP

The chemical identities of the p-NP and physical and chemical characteristics of the p-NP are presented, in Tables 2.1 and 2.2, respectively (Public Health Service, 1992).

Table 2.1 The chemical identities of the p-NP (Public Health Service, 1992).

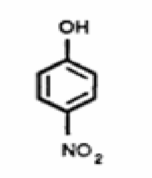
Characteristics	**p-Nitrophenol**
Chemical name	4-Nitrophenol
Chemical formula	$C_6H_5NO_3$
Synonyms	4-hydroxynitrobenzene, p-NP
Chemical structure	
EPA hazardous waste identification number	U170

Table 2.2 The physical and chemical characteristics of the p-NP (Public Health Service, 1992).

Property	**p-Nitrophenol**
Molecular weight	139.11 g /mol
Color	Colourless or light yellow
Physical state	Crystalline solid
Melting point	113-114°C
Boiling point	297 °C
Density at 20 °C	1.27 mm Hg at 20 °C
Water solubility	8.04 g/l at 15 °C 16 g/l at 100 °C
Oder	Slight oder
Solubility	Distilled water: 16.000 mg/l at 25 °C Sea water: 10.795 mg/l at 20 °C Organic solvent: toluene, ethanol, chloroform, ethyl ether and alkali hydroxides
Partition coefficients	Log octanol/water: 1.91 Log K_{oc}: 2.18-2.42
Vapour pressure	0.0003 mm Hg at 30 °C
Henry's law constant	3.5×10^{-9} atm-m^3/mol at 25-30 °C

2.1.1.2 Use and Environmental Concerns of Nitrophenol

Nitrophenols are produced by microbial hydrolysis of several organophosphorous pesticides such as parathion or by photodegradation of pesticides' (Haghighi-Podeh, Bhattacharya, & Qu, 1995; Karim & Gupta, 2001; Uberoi & Bhattacharya, 1997). Nitrophenols exist in three possible isomeric form based on the position of the nitro group on the phenolic ring, called ortho-, meta- and para-nitrophenol. These commonly known as 2-,3- and 4-nitropnenol or meta, ortho and para-nitrophenol, respectively. Among the nitrophenol: "2-nitrophenol (2-NP), 4-nitrophenol (4-NP) and 2,4-dinitrophenol (2,4-DNP) are listed by the Environmental Protection Agency (EPA)'s as Priority Pollutants". Furthermore, the EPA recommends restricting their concentrations in natural waters below 10 ng/l" (EPA, 1980).

Since these chemicals are frequently used for industrial, agricultural, and defense purposes, usually they find their way into the effluents from these sources. These compounds have significant health risks since they have mutagenic and carcinogenic activity and may bioaccumulate in the food chain and receving waters (Donlon, Razo-Flores, Lettinga, & Field, 1996; Karim & Gupta, 2001). The EPA has also set the pretreatment standards for discharge of nitrophenols applicable to discharges of manufacturing synthetic fibers, thermoplastic resins, commodity organic chemicals, bulk organic chemicals and specialty organic chemicals. The pretreatment standards for nitrophenols are listed in Table 2.3 (EPA, 1987).

Table 2.3 Effluent Discharge Standards for Nitropenols (EPA, 1987).

Compounds	**Treatment standarts (µg/l)**	
	Maximum for any one day	**Maximum for montly avarage**
For point sources discharging into POTW's		
2-nitrophenol	231	65
4-nitrophenol	576	162
For direct discharge point sources that use end of pipe biological treatment		
2-nitrophenol	69	41
4-nitrophenol	124	72
2,4-dinitrophenol	123	71
For direct discharge point sources that do not use end of pipe biological treatment		
2-nitrophenol	231	65
4-nitrophenol	576	162
2,4-dinitrophenol	4291	1207

POTW= Publicly Owned Treatment Works

2.1.2 Nitrobenzene

2.1.2.1 The Physical and Chemical Characteristics of Nitrobenzene (NB)

The chemical identities of the NB and physical and chemical characteristics of the NB is presented in Table 2.4 and 2.5, respectively (EPA, 1995).

Table 2.4 The chemical identities of the NB (EPA, 1995).

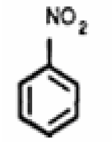
Characteristics	**Nitrobenzene**
Chemical name	Nitrobenzene
Chemical formula	$CHNO_2$
Physical state	Liquid
Synonyms	nitrobenzol; oil of mirbane
Chemical structure	

Table 2.5 The physical and chemical characteristics of the NB (EPA, 1995).

Property	**Nitrobenzene**
Molecular weight	123.06 g /mol
Boiling point	210.9 °C at 1 atm
Water solubility	1.9 g/l at 20 °C; 2.1 g/l at 25 °C
Vapor pressure	0.15 mm Hg at 20 °C; 0.27 mm Hg at 25°C
Henry's law constant	2.3 x 10^{-5} atm-m^3/mole at 25°C
K_{oc}	36-650 l/kg
Log K_{ow}	1.85 l/kg
Flash point	88°C

K_{oc}: organic carbone coefficient; K_{ow}: Octanol/water partition coefficient

2.1.2.2 Use and Environmental Concern of NB

Nitrobenzene (NB) is a synthetic compound, not occur naturally. More than 95% of nitrobenzene is used in the production of aniline. A few amount is used in the manufacture of rubber chemicals, pesticides, insecticide, wood, petroleum, dyes, soap, shoe, floor polishes, leather dressings, paint solvents and pharmaceuticals (Ni, Wang, & Kokot, 2001; EPA, 1995). Furthermore nitrobenzene is also used as a solvent in petroleum refining and in the manufacture of cellulose ethers and acetates, in the manufacture of dinitrobenzenes and dichloroanilines, and in the synthesis of other organic compounds, including acetaminophen (EPA, 1995). Early in the 20th century, nitrobenzene had some use as a food additive as well as extensive use as a solvent in various proprietary products, including boot polish, inks (including inks used for stamping freshly laundered hospital baby diapers) and several disinfectants (EPA, 1995).

NB is relatively toxic and persistent in the environment. It and is listed as a priority pollutant by the U.S. EPA (Majumder & Gupta, 2003). If the concentration exceeds 2 mg/l in the wastewaters, it is declared to be hazardous waste. Because of its toxicity, nitrobenzene has been placed as one of the 129 priority pollutants by the U.S. EPA (Majumder & Gupta, 2003). NB is a potential atmospheric pollutant due to its volatile structure. Nitrobenzene has been observed in air samples taken from the urban, rural, and waste disposal areas in New Jersey in 1982 (ATSDR, 1990).

Available information on environmental effect indicates that NB is moderately toxic to aquatic life (EPA, 1985). Ninety-six-hour LC values for *Lepomis macrohirus* (bluegill sunfish), *Pimephales promelas* (fathead minnow), *Brachydanio rerio* (zebrafish) were 117,112.5 and 58.6 mg/l NB (EPA, 1985).

2.1.3 Biodegradation of Nitroaromatic Compounds

The nitro group exists as a resonance hybrid because of that oxygen atoms are more electronegative than the nitrogen atom. Therefore, the most common reaction of the nitro group in the biological system is reduction (Spain, 1995). In addition, iron (II), other metals and reduced sulfure compounds can serve as nitro group and amino group which are relatively stable (Spain, 1995). Nitro group and amino group are relatively stable. However the nitroso and hydroxilamino groups are elecrophiles that can interact with biomolecules to cauce toxic, carcinogenic and mutagenic effects (Spain, 1995).

The reduction process can proceed either by one-electron (oxygen-sensitive) or two-electron (oxygen-insensitive) reduction of nitro group (Spain, 1995). The reactions of one and two electron reduction of the nitro group are depicted in Figure 2.1. The one-electron reduction of the nitro group produces a nitro radical anion, which can be oxidized by molecular oxygen. Anaerobic bacteria such as Clostridium spp, facultative bacteria such as Escherichia coli and Entrobacter spp. can catalyze one-electron reduction of nitro group. In contrast, the sequential two electron reduction of nitro group results in the formation of either hydroxylamino or amino groups as a product by adding electron pairs donated by reduced pyridine nucleotides. This kind of nitro reductase is termed an oxygen-insensitive reductase. The reaction pathway includes the nitroso derivates, which so reactive and unstable. Nitroso and hydroxylamino intermedies are much toxic and carcinogenic (Spain, 1995).

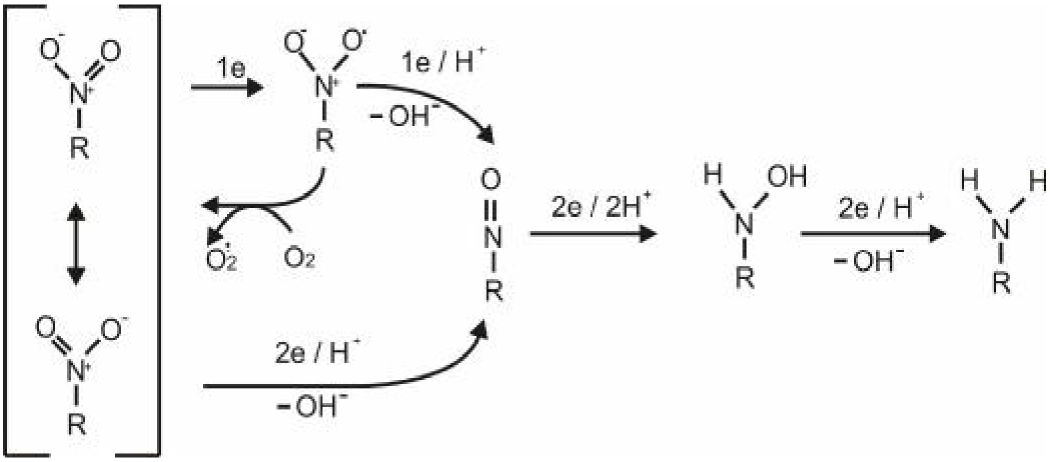


Figure 2.1 Reduction of nitro groups by one-electrone or two electrone mechanisms.

2.1.3.1 Biodegradation of p-Nitrophenol (p-NP)

Gorontzy, Kuver, & Botevogel (1993) showed that p-NP is transformed partially or completely to its amino derivatives under anaerobic conditions. Boyd, Shelton, & Bery (1983) investigated the mineralization of phenolic compounds in digested sludge. After an initial lag phase of 8 weeks, p-NP transformed to the less toxic para-aminophenol (p-AP) and further mineralized to methane and carbon dioxide gas in anaerobic digested sludge. Ninety-five percent of p-NP was recovered as CH_4 (42%) and CO2 (58%), which demonstrated a complete conversion of p-NP to methane and carbone dioxide. Melgoza & Buitron (2001) found the transformation of p-NP to p-aminophenol (p-AP) under anaerobic conditions with removal efficiencies near to 100%. A mineralization of 100% of p-AP was found in the aerobic stage. Donlon et al., (1996) investigated the anaerobic transformation and degradation of nitrophenols in a continuously fed upflow anaerobic sludge banked (UASB) reactor. It was found that p-NP converted to p-AP under anaerobic conditions.

2.1.3.2 Biodegradation of Nitrobenzene (NB)

Three different mechanisms have been described for the mineralization of NB. The first involves its degradation via catechol to 2-hydroxymuconic semialdehyde by *Comamonas sp.* in an oxidative pathway (He & Spain, 1999; Nishino & Spain, 1995). In the second mechanism, reductive pathway was described with Pseudomonas pseudoalcaligenes (He & Spain, 1999; Nishino & Spain, 1993). NB is degraded by Pseudomonas pseudoalcaligenes JS45 to 2-aminomuconic semialdehyde via 2-aminophenol, which is further degraded to pyruvate and acetaldehyde. The third mechanism is reported under anaerobic conditions. NB is reduced to aniline in an anaerobic or acidogenic reactor (Aziz, Ng, & Zhou, 1994; Zhao & Ward, 1999). Aziz, Ng & Zhou, (1994) investigated the biodegradability of nitrobenzene using sequential acidogenic-aerobic process. The results of NB degradation show that 1 mol of NB was reduced to 1 mol of aniline in the acidogenic process. Aniline was rapidly biodegraded in aerobic process. Peres, Naveau & Agathos, (1998) investigated the biodegradation of NB to aniline in a single reactor. The behavior of the microbial population was indicated that NB was reduced to aniline by the reductive consortium in the first step, and, in the second, oxidative step, aniline was mineralized to catechol and 2-hydroxymoconic semialdehyde.

2.1.4 Anaerobic Biological Treatment

Anaerobic biological treatment systems may have promising applications for the removal of nitroorganic pollutants because the anaerobic microorganisms are able to degrade these compounds (Donlon et al., 1996). The conjugation of unstable nitroso and hydroxylamine intermediates results into the formation of complex azo or azoxy compounds under aerobic conditions. The high electron withdrawing properties of the nitro group is the main factor that makes the recalcitrant to the oxidative cleavage of the aromatic ring during aerobic processes (Melgoza & Buitron, 2001). However, under anaerobic conditions, nitrophenols readily transformed to their corresponding amines. On average, aromatic amines are 500-fold less toxic than their corresponding nitroaromatics. This suggests that anaerobic conditions detoxify the nitrophenolic wastewater (Donlon et al., 1996; Karim, & Gupta, 2003).

Combined anaerobic/aerobic processes are a viable alternative for the treatment of xenobiotic compounds which are difficult to treat by traditional processes (Speece, 1996). The mineralization of some recalcitrant pollutants has been possible by using the sequential anaerobic/aerobic treatments (Speece, 1996). Generally, aerobic polishing step is needed after the anaerobic system (pretreatment) to meet the effluent quality standards (Angenent & Sung, 2001).

2.1.5 Literature Review for the Treatment of p-Nitrophenol (p-NP)

Different reactor processes were used to treat the nitrophenolic wastewaters: anaerobic biodegradation process such as continuously fed upflow anaerobic sludge banked (UASB) reactor (Donlon et al., 1996 ; Karim & Gupta, 2001), anaerobic biological fluidized bed reactor (Tseng & Yang, 1994) and aerobic biodegradation process such as activated sludge units (Bhatti, Toda, & Furukawa, 2002). Moreover, the degradation of p-NP was investigated in a batch biofilter under sequential anaerobic/aerobic environments (Melgoza & Buitron, 2001).

The anaerobic transformation and degradation of nitrophenols was investigated in a continuously fed upflow anaerobic sludge blanked (UASB) reactor seeded with granular sludge with a volatile fatty acid (VFA) mixture as the primary substrate (Donlon et al., 1996). High p-NP removal efficiencies (>99%) were obtained at influent VFA concentration of 3.76 gCOD/l at a HRT of 0.33 days. The VFA removal efficiency was up to 99%. The VFA conversion to CH_4 was found as 89%. Through experimental period, p-NP was converted to p-AP under anaerobic condition. Another study on the treatment of NP in UASB reactor was carried out by Karim & Gupta (2001). Overall removal of 2,4-NP was always more than 99% at HRTs varied between 12h and 30h. p-AP was found as the main intermediate.

Melgoza & Buitron, (2001) studied the degradation of p-NP in a anaerobic/aerobic process combined into a single reactor. A pilot biofilter was used for the experiment. After 230 days of operation, the p-NP removal efficiency was 98% through reaction time of 11.5 h (8 h for the anaerobic phase and 3.5 h for the aerobic one) and the p-NP was transformed to p-AP in the anaerobic phase. A mineralization of 100% of p-AP was found in the aerobic stage.

Bhatti, Toda, & Furukawa (2002) investigated the treatment of p-NP under facultative aerobic conditions. 500 mg p-NP/l was completely degraded at a hydraulic retention time of 11 h.

2.1.6 Literature Review for the Treatment of Nitrobenzene (NB)

Treatment of NB was carried out using an anaerobic-aerobic reactor system by Aziz, Ng, & Zhou, (1994). The experiments were conducted continuously in two parallel sets. The system was operated at various HRTs (8, 12, 16 and 24 days) and influent NB concentrations (50, 100, 150 and 200 mg/l).The removal efficiency of nitrobenzene was between 75% and 85% when the wastewater was treated by the aerobic process alone. However, the removal efficiency of NB was more than 95% in the acidogenic-aerobic process. The studies shown that NB was converted to aniline during the acidogenic process and the aniline rapidly mineralized in the subsequent aerobic process.

The treatment performance of wastewater containing NB was investigated in a hybrid reactor (Majumder & Gupta, 2003). The hybrid reactor was consisted of a trickling filter (TF) and an aeration tank (AT) giving a combination of attached growth and suspended growth systems. The study of hybrid reactor's performance was obtained at five different HRTs (4.4, 5.6, 8.5, 9.25 and 11.9 h). A removal of 60–95.8 % COD and 80–90.23% NB was observed during the acclimation period. Maximum COD and NB removal efficiencies were 95.83% and 97.93%, respectively at a HRT of 29.55 h. A loss of 9.44% NB was observed due to volatilization while the biological removal of NB was found as 87.49%.

Bell, Devlin, Gillham & Binning, (2003) studied the remediation of NB contaminated ground water in a sequential anaerobic and aerobic biodegradation treatment system under laboratory conditions. Zero valent granular iron zone reduce the NB to aniline and a passive oxygen release occurs from the aerobic biodegradation of the aniline. In batch laboratory experiments, NB was found to reduce to aniline quickly in the presence of granular iron. Aniline was found to be readily biodegraded under aerobic conditions.

2.1.7 Literature Review for the Anaerobic Baffled Reactor (ABR)

Anaerobic baffled reactor (ABR) is high-rate and compartmentalise reactor containing between 3 and 8 compartments (Barber & Stuckey, 1999). Anaerobic Baffled Reactor (ABR) was developed when high biomass concentration (>20 g/l) could not removed in the rotating discs into anaerobic Rotating Biological Contactor (RBC) (Barber & Stuckey, 1999).

ABR reactor consists of a series of baffles to forces the wastewater to flow from inlet to outlet. The flow is under and over the baffles. During upflow, wastewater contact with the active biomass. The ABR can be described as a series of upflow anaerobic sludge blanked reactor (UASB) (Barber & Stuckey, 1999).

As the anaerobic baffled reactor (ABR) has been compared with traditional anaerobic reactors include higher resilience to hydraulic and organic shock loads, longer biomass retention times and lower sludge yields. There are no requirement unusual settling properties for biomass. The advantages of ABR reactor are summarized in Table 2.6 (Barber & Stuckey, 1999). The most significant advantage of ABR is its ability to separate acidogenesis and methanogenesis. The reactor behaves as a two-phase system. This design characteristic permits separation of more sensitive anaerobic population such as methanogens. The separation of acetogenic and methanogenic phases increase in protection against toxic materials and causes higher resistance to changes in environmental parameters such as pH, temperature and organic loading (Barber & Stuckey, 1999).

Hutnan et al., (1999) comprised startup of UASB reactor, hybride reactor and anaerobic baffled reactor (ABR). The wastewater contained sodium acetate and glucose giving a 6000 mg/l of COD. Organic loading rate was increased gradually from 0.5 to 15 kg/m^{3}day in all reactors. As reactor's performances were compared, the lower biomass wash out was observed from the ABR. Furthermore, biomass granulation was faster in the ABR than that in the other two reactors. The demand of sodium bicarbonate for pH maintenance in ABR was two times higher from the UASB and anaerobic hybride reactor (AHR). Thus indicated that the ABR was superior compared to UASB and AHR reactors.

Table 2.6 Advantages of the anaerobic baffled reactor (ABR) (Barber & Stuckey, 1999).

Construction
Simple design
No moving parts
No mechanic mixing
Inexpensive construction
High void volume
Reducing clogging
Reduced sludge bed expension
Low capital and operating costs
Biomass
Unusual settling properties does not require for the biomass requirement
Low sludge generetion
High solids retention times
Retention of biomass does not require a solid-settling chamber
No special separation required for gas and sludge
Operation
Intermitten operation is possible
Extremely stable to hydraulic shock loads
Protection from toxic materials in influent
Long operation times without sludge wasting
High stability to organic shocks

The chamber number of ABR is important to treat the wastewater. Boopathy, (1998) investigated the effect of chambers of ABR on the treatment of swine wastes. Four lab-scale anaerobic baffled reactors with respectively two, three, four and five chamber were used for the treatment of swine wastes. The additional chambers in an ABR make a significant difference to particle retention. In the four and five chambers ABRs were observed more gas productions compared with the two and three chamber ABRs. Based on this study, four or five chambers of ABR were commended for the most efficient treatment of swine wastes and maximum gas production Boopathy, (1998).

The effect of recycle on the efficiency of ABR are unclear. Theoretically, recycle should have a negative effect on reactor hydrodynamics by causing increased mixing. Mixing by recycle caused a return to single phase digestion. Therefore, the benefits arising from the separation of the acidogenic and methanogenic phases are lost and removal effiency reduce because the reactor approaches a completely mixed system (Barber & Stuckey, 1999). However, the recycle of the effluent also was observed a positive effect such as increasing the methane yield, protecting the problems of low pH at the first compartment of the reactor and reducing the substrate inhibition in the influent (Barber & Stuckey, 1999). Setiadi, Husaini, & Djajadiningrat, (1996) investigated the effects of recycle on anaerobic baffled reactor treating palm-oil mill effluent wastewater. With a recycle of effluent to influent, the alkalinity was recovered from the effluent. Therefore, the influent COD concentration and alkalinity requirement reduced due to recycle. The stability of the system was achieved at high recycle ratio. A recycle more than 15 times per 71 h kept the system pH higher than 6.8 without alkalinity supplementation. Consequently, the using of recycle depends on the type of waste being treated. If the pH problem is severe, the influent contains high levels of toxic material, or high loading rates are preferred, recycle should be beneficial (Barber & Stuckey, 1999).

Start-up procedure is important in anaerobic treatment systems. A successful start-up allows the acclimation and phase separation of bacteria in the reactor. Once the biomass has been established, either as a granular particle or a floc, while the reactor operation is quite stable (Speece, 1996). Initial loading rates should be low so that slow growing micro-organisms are not overloaded. The recommended initial loading rate is 1.2 kg COD/m^{3}day, however, successful start-up of a pilot scale ABR has been achieved at significantly higher primary loading rates such as 4.33 kgCOD/m^{3}day (Barber & Stuckey, 1996). During the start-up of the ABR, potential problems such as total volatile fatty acid accumulation and low pH can arise. These can soluble with provide sufficient substrates in the final compartments during start-up period. This are achieved to the development of the split fed in the ABR as reported by Sallis & Uyanik, (2003). To prevent the accumulation of VFA during the start-up, the feed was split between the compartments, which would result in a longer HRT, and SRT in the initial compartments and obtained enough substrate for the microorganisms in the final compartments.

Sulfide production in an anaerobic reactor treating industrial wastewaters can cause operating problems. Sulfate-reducing bacteria can out compete with methanogens for available substrate, and hydrogen sulfide production can be predominant over methane gas production. In this case, organic carbone is oxidized to carbone dioxide with reduction of sulfate to hydrogen sulfide. Therefore, methane production may be limited (Malina & Pohland, 1992). Vossoughi, Shakeri & Alemzadeh, (2003) investigated the effect of the COD/SO_4 ratio on the performance of an anaerobic baffled reactor (ABR) treating synthetic wastewater containing molasses giving a COD of 3000 mg COD/l. The results of this study indicated that when COD/SO_4 ratios were decreased from 16.7 to 6.0, COD conversion was not decreased, but a slight increase in COD removal was observed. Maximum COD and sulphate removal were found as 86% and 97% at a HRT of 1 day, respectively. COD removal efficiencies varied between 84% and 86% at a HRT of 1 day with decreasing COD/SO_4 ratios. When COD/SO_4 ratios were below 6.0 inhibition was not observed. Fox & Venkatasubbiah, (1996) investigated the effects of sulphate reduction in the ABR by treating a sulphate containing pharmaceutical wastewater. At steady-state, 50 % COD removal and 95 % sulphate reduction was found at HRT of 1 day. When COD/SO_4 ratio decreased from 150/1 to 24/1, sulphate removal reduced from 95 % to 50 %. Increasing sulphate concentrations showed inhibition of sulphate reduction due to elevated sulphide concentrations.

There are several studies performed with ABR treating the different wastewaters such as whisky distillery wastewater (Akunna & Clark, 2000), textile dye wastewater (Bell & Buckley, 2003), decolorization of dyes (Bell, Plumb, Buckley & Stuckey, 2000), nitrogen containing wastewaters (Bodik, Kratochvil, Gasparikova & Hutnan, 2003), swine wastewater (Boopathy,1998), domestic wastewater (Dama et al., 2002), pulp and paper mill black liquor (Grover, Marwaha, & Kennedy, 1999), palm oil mill effluent wastewater (Setiadi, Husaini, & Djajadiningrat, 1996), and ice-cream wastewater (Uyanik, Sallis, & Anderson, 2002).

Akunna & Clark, (2000) investigated the performance of anaerobic baffled reactor in the treatment of a whisky distillery wastewater. The ABR was fed with whisky distillery wastewater containing 9500 mg/l COD at different HRTs (2, 4, 7 and 10 days). Up to 80% COD (or 90% BOD) removal was observed at a HRT of 2 days. Biogas production rate increased from 10 to 22 l/day with decreasing of HRT from 10 to 2 days. The methane content of the biogas varied between 60% and 70% throughout the experiment.

Bell & Buckley, (2003) investigated the decolorization of the dye CI Reactive Red 141 in a laboratory-scale anaerobic baffled reactor (ABR). 90% COD and %86 color removal efficiencies were found at COD loading rate of 4.8 g/l.day and HRT of 20 h, respectively. In another study, decolorization of the 250 mg/l of tartrazine dye was investigated in a laboratory-scale anaerobic baffled reactor (Bell et al., 2000). 50–60% COD removal and 95% color reduction were achieved in ABR at a HRT of 20 h. An industrial wastewater from a food dye manufacturer was also investigated in the same study. 70% organic matter removal and 90% color reduction were achieved in ABR.

The removal of the NH_4-N was investigated in a treatment system consisted of an anaerobic baffled filter reactor and the following aerobic post treatment (Bodik et al., 2003). The HRTs in anaerobic and aerobic parts of the reactors were about 15 and 4 h, respectively. 78.6–83.0 % COD, 92.5–94.0% BOD_5 and 80.9–92.7% suspended solid (SS) were removed. The removal of the NH_4-N varied between 46.4% and 87.3%. In another study, ABR was used to biological treatment of swine wastes (Boopathy, 1998). 70 -78% COD removal efficiency was found at an HRT of 15 days. Total Kjheldhal and ammonia nitrogen removal were found between 48-55% and 38-44%, respectively.

Grover, Marwaha, & Kennedy, (1999) investigated the effect of different pH, temperatures, hydraulic retention times and organic loading rates on an anaerobic baffled reactor (ABR) treating black liquor from pulp and paper mills. A maximum COD reduction was found as 60% at HRT of 2 day.

The stability and performance of an anaerobic baffled reactor (ABR) treating an ice-cream wastewater was investigated at HRTs varied between 0.43 and 10 days (Uyanik, Sallis, & Anderson, 2002). COD removal efficiency was found as 99% at all HRTs. High COD removal efficiency in ABR came from its compartmentalized structure. The most of the influent COD was removed in compartment 1 (approximately 80%) through study.

Application of a pilot-scale anaerobic baffled reactor was realized by Dama et al., (2002). Pilot-scale anaerobic baffled reactor (ABR) with a volume of 3200 l was used for the treatment of domestic wastewater at South Africa. The pilot scale reactor has 8 compartments. The HRT in the reactor was decreased from 60 h to 20 h. The COD removal efficiency was found below 60% at a HRT of 60 h. The removal efficiency increased to 80% when the HRT was decreased to 32h. COD removal efficiencies were between 70% and 90% at a HRT of 20 h. Pathogen removal in the ABR is possible due to the long solids retention times. Total coliforms and *Escherichia coli* (*E.coli*) in the effluent of ABR was found as 43.000 cfu/100 ml and 7.000 cfu/100 ml, respectively.

2.1.8 Literature Review for Anaerobic Migrating Blanked Reactor (AMBR)

The anaerobic migrating blanket reactor (AMBR) was developed as high rate anaerobic reactor with compartmentalisation, continuous flow and simple design (Angenent & Sung, 2001). The AMBR is a flow-through reactor consisting of three to five compartments. It is operated without a hydraulic upflow pattern for mixing and granular sludge development, which eliminates gas-solid separation and the feed distribution systems. The flow is reversed periodically to prevent the biomass accumulating in the final compartment. Because of this approach the biomass was able to migrate with the flow over the horizontal plane of the system. This promoted the granulation and eventually gave the AMBR its name. With reversing the flow, the final compartment is the first or final compartment according to whether the flow is reversed or not. The flow over the horizontal plane of the reactor is reversed one a week. A weekly change in flow direction was chosen to prevent of the phase separation, to prevent a pH drop due to TVFA accumulation in the initial compartment and to prevent the biomass levels due to anticipated biomass migration between compartments (Angenent, Banik & Sung, 2001). Hence effluent recycling is not required to control pH in the initial compartments. This is advantageous because effluent recycling changes the compartmentalized reactor from a system that approaches plug flow conditions into a system that approaches completely mixed conditions, which tends to reduce removal efficiencies (Angenent & Sung, 2001; Angenent, Banik, & Sung, 2001).

The performances of AMBR, UASB and anaerobic sequencing batch reactor (ASBR) were compared in terms of stabilization of organic matter **(**Angenent & Sung, 2001). The AMBR was very efficient with COD removals of 94.9% at a hydraulic retention time (HRT) of 12 h comparing with other reactors. The AMBR could be operated at higher loading rates such as 22.6 g/lday comparing with other reactors. The specific methane production rate (SMPR) in AMBR was higher (5.4 l/day) than the others. The granulation was very well. Furthermore, because of good granulation in AMBR, filamentous bacteria were not observed compared to UASB and ASBR. Thus, it found that the AMBR was superior to the UASB reactor and the ASBR in terms of maximum COD loading rates and SMPR (Angenent & Sung, 2001).

The effect of an organic shock load was investigated on the performance and stability of a laboratory scale AMBR (Angenent, Abel, & Sung, 2002). The volumetric loading rate (VLR) was increased from 27 to 50 g COD/l day at constant hydraulic retention time of 42 h during study. The pH levels stayed favorable and biomass washout was limited during the shock load due to the damping effects of a compartmentalized configuration. During the shock load, the propionate production in the initial compartments of the AMBR remained at the same level as before the shock load, while the acetate production rose sharply. Because propionate is the most difficult volatile fatty acid to be removed, unstable conditions due to excessive propionate accumulation during the shock load were prevented.

Structure and activity of microbial community in a compartments of AMBR was investigated by Angenent, Zheng, Sung & Raskin, (2000). Researches indicated that biomass staging in the AMBR was higher in the middle compartment compared with the outside compartments. Long-term overload conditions resulted in too much biomass staging, and low levels of acetate-utilizing methanogens in the outside compartments diminished acetate degradation in the final compartment. This was demonstrated that recycling of biomass between compartments was important to maintain sufficient levels of acetate-utilizing methanogens in the outside compartments.

The studies related to the treatment of industrial wastewater in AMBR are scarcely. Angenent & Sung, 2001 and Angenent, Abel, & Sung, 2002 were determined the performance of a laboratory-scale AMBR using a synthetic wastewater containing sucrose and and non-fat dry milk as substrate.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental System

3.1.1 Anaerobic Migrating Blanked Reactor (AMBR)/Completely Stirred Tank Reactor (CSTR) System

A schematic of the lab-scale sequential AMBR and CSTR reactors used in this study is presented in Figure 3.1. A continuously fed stainless steel anaerobic AMBR and an aerobic CSTR reactor were used in sequence for the experimentation. The effluent of the anaerobic AMBR reactor was used as the influent of aerobic CSTR reactor. The AMBR reactor consisted of a rectangular (inside dimensions: length = 45 cm, height = 20 cm, width = 15 cm) with an active volume of 13.5 l which was divided into three compartments. Round openings with a diameter of 2.5 cm from the backside of the stainless steel sheets separated the compartments. These openings were placed at the bottom to create sufficient contact between biomass and substrate. Three compartments were mixed equally every 15 min at 60 rpm to ensure gentle mixing. The flow over the horizontal plane of the reactor was reversed once a week. A weekly change in flow direction was chosen to prevent a the pH drop due to VFA build up in the initial compartment and to prevent unequal biomass levels due to anticipated biomass migration between compartments. The samples were withdrawn from the AMBR reactor after stopping the mixing process for 15 min. The influent feed was pumped using peristaltic pump. The outlet of AMBR was connected to a glass U-tube for controlling the level of wastewater. The produced gas was collected via porthole in the top of reactor. The operating temperature of the reactor was maintained constant at 37±1 ◦C by placing the AMBR reactor on a heater. A digital temperature probe located in the middle part of the second compartment provided the constant operation temperature. This provided a homogenous temperature in whole compartments of AMBR reactor. The aerobic CSTR reactor consisted of an aerobic (effective volume=9 l) and a settling compartment (effective volume = 1.32 l).

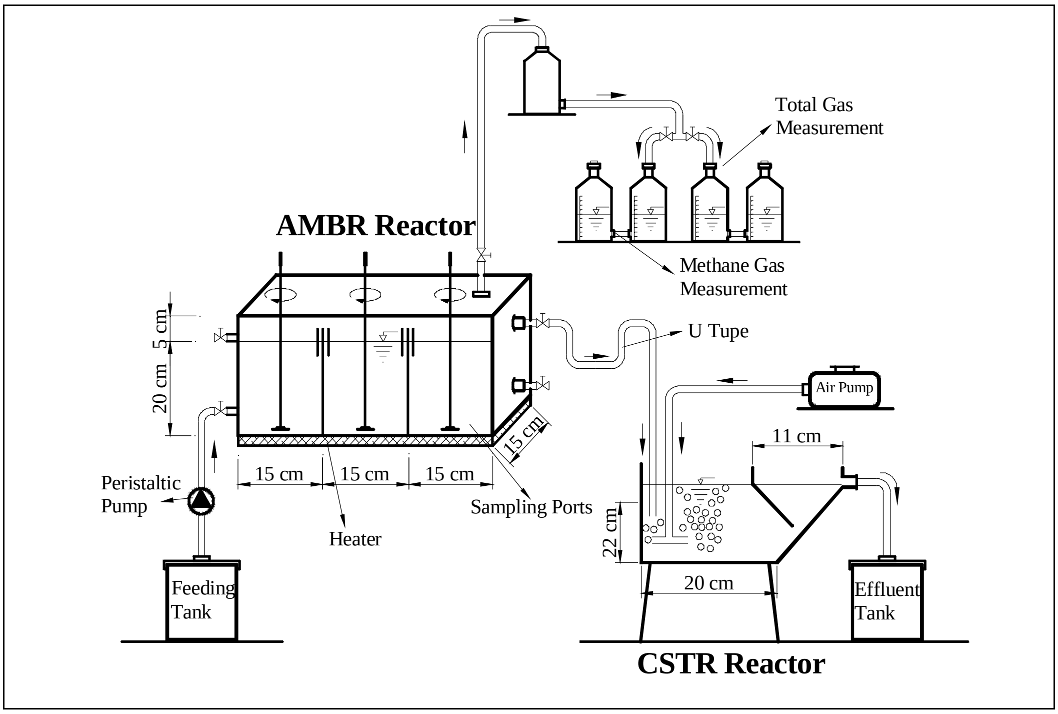


Figure 3.1 Schematic configuration of lab-scale anaerobic (AMBR)/aerobic (CSTR) sequential reactor system.

3.1.2 Anaerobic Baffled Reactor (ABR)/Completely Stirred Tank Reactor (CSTR) System

A schematic of the lab-scale sequential ABR and CSTR reactors used in this study is presented in Figure 3.2. The effluent of the anaerobic ABR reactor was used as the influent of aerobic CSTR reactor. The ABR reactor was rectangular box having the dimensions 20 cm wide, 60 cm long and 40 cm high. The ABR reactor with the active reactor volume (38.4 l) was divided into four equal compartments by vertical baffles. Only three compartments were used throughout this study (effective volume =28.8 l). The last compartment was used as settling tank. Each compartment was further divided into two by slanted edge (45°C) baffles to encourage mixing within each compartment. Therefore, down-comer and up-comer regions were created. The liquid flow is alternatively upwards and downwards between compartment partitions. This provided effective mixing and contact between the wastewater and biomass at the base of each upcomer (Wang, Huang & Zhao, 2004). In other words, during upflow, the waste flow contact with the active biomass and it is retained within the reactor providing a homogenous distribution of wastewater. An additional mixing was not supplied to the compartments of the reactor. The width of the downcomer was 4 cm and the width of the up-comer was 11 cm. The passage of the liquid from each compartment to another was through an opening with size 40 mm×10 mm which located about 80 mm from the top of each compartment. The liquid sampling ports were located at 40 mm back of the effluent opening of each compartment. The sludge sampling ports were also located in the center of the compartments and 80 mm above from the bottom of the each compartment. The influent feed was pumped using a peristaltic pump. The outlet of ABR was connected to a glass U-tube for controlling the level of wastewater. The produced gas was collected via porthole in the top of the reactor. The operating temperature of the reactor was maintained constant at 37±1 ◦C by placing the ABR reactor on a heater. A digital temperature probe located in the middle part of the second compartment provided the constant operation temperature. This provided a homogenous temperature in whole compartments of ABR reactor. The aerobic CSTR reactor consisted of an aerobic (effective volume=9 l) and a settling compartment (effective volume = 1.32 l).

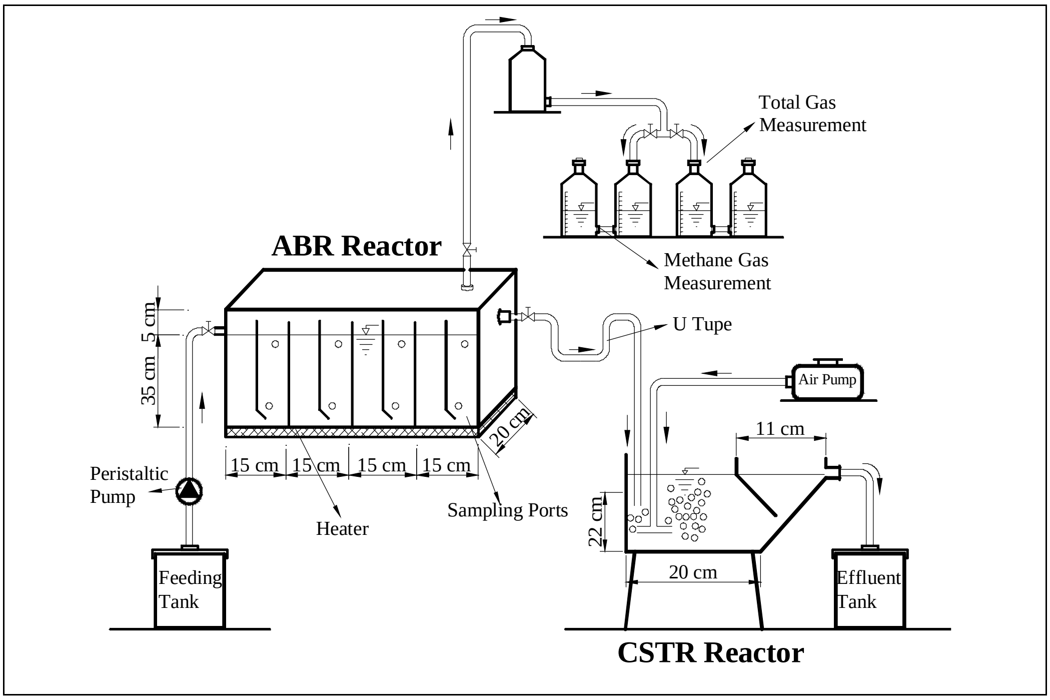


Figure 3.2 Schematic configuration of lab-scale anaerobic (ABR)/aerobic (CSTR) sequential reactor system.

3.2 Seed of Reactors

Partially granulated anaerobic sludge was used as seed in AMBR and ABR reactors. The seed sludge was obtained from an anaerobic upflow anaerobic sludge blanket reactor containing acidogenic and methanogenic partially granulated biomass taken from the Pakmaya Yeast Beaker Factory in Izmir, Turkey. Activated sludge culture was used as seed for the aerobic CSTR reactor and it was taken from the activated sludge reactor of Pakmaya Yeast Beaker Factory in Izmir. The volatile suspended solid (VSS) concentration of seed sludge in AMBR and ABR reactor were adjusted as 25 g/l and 30 g/l, respectively. The mixed liquor solids concentration (MLSS) in the every two CSTR were adjusted between 2000 and 3000 mg/l. The sludge volumes added to AMBR, ABR and CSTR reactors were 3 l, 7 l and 2 l, respectively.

3.3 Composition of Synthetic Wastewater

p-NP concentrations varying between 10 and 400 mg/l and NB concentration varying between 10 and 700 mg/l were used through continuous operation of the AMBR and ABR reactors. Glucose was used as primary substrate giving a COD concentration of 3000 ± 100 mg/l. Vanderbilt mineral medium was used in synthetic wastewater as mineral source. This mineral medium consisted of the following inorganic composition (in mg/l): NH_4Cl, 400; $MgSO_4.7H_2O$, 400; KCl, 400; $Na_2S.9H_2O$, 300; $(NH_4)_2HPO_4$, 80; $CaCl_2.2H_2O$, 50; $FeCl_3.4H_2O$, 40; $CoCl_2.6H_2O$, 10; KI, 10; $(NaPO_3)_6$, 10; L-cysteine, 10; $AlCl_3.6H_2O$, 0.5; $MnCl_2.4H_2O$, 0.5; $CuCl_2$, 0.5; $ZnCl_2$,0.5; NH_4VO_3, 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 (Speece, 1996). The anaerobic conditions were maintained by adding 667 mg/l of Sodium Thioglycollate (0.067 %) which is proposed between 0.01-0.2 % (w/w) for maintaining the strick anaerobic conditions (Speece, 1996). The alkalinity and neutral pH were adjusted by addition of 5000 mg /l $NaHCO_3$.

3.4 Analytical Methods

3.4.1 Dissolved Chemical Oxygen Demand (DCOD) Measurement

The dissolved COD was measured calorimetrically by using closed reflux method (APHA AWWA, 1992). Firstly the samples were centrifuged 10.0 min at 7000 rpm. Secondly, 2.5 ml samples were mixed with 1.5 ml 10216 mg/l $K_2Cr_2O_7$, 33.3 g/l $HgSO_4$ and 3.5 ml 18 M H_2SO_4 containing 0.55% (w/w) Ag_2SO_4. Thirdly the closed sample tubes were stored in a heater with a temperature of 148°C for two hours. Finally, after cooling, the samples were measured at a wave-length of 600 nm with a Pharmacia LKBNovaPec II model spectrophotometer. The COD values given in Tables and in Figures are measured as dissolved COD (DCOD).

3.4.2 Gas Measurements

Gas productions were measured with liquid displacement method. The total gas was measured by passing it through a liquid containing 2% (v/v) H_2SO_4 and 10% (w/v) NaCl (Beydilli, Pavlosathis & Tincher, 1998). Methane gas was detected by using a liquid containing 3% NaOH to scrub out the carbon dioxide from the biogas (Razo-Flores et al., 1997). The methane gas percentage in biogas was also determined by Dräger Pac®Ex methane gas analyzer. The H_2S gas was measured using Dräger (Stuttgart, Germany) kits in a Dräger H_2S meter. H_2 gas was measured using (Dräger Pac®Ex) H_2 meter. N_2 gas was measured by discarding of the sum of $CH_4 + H_2S + H_2$ gases from the total gas.

3.4.3 Mixed Liquor Suspended Solids (MLSS), Mixed Liquor Volatile Suspended Solids (MLVSS), Suspended Solids (SS) and Volatile Suspended Solids (VSS) Measurements

Biomass was measured as total suspended solid (TSS) and volatile suspended solid (VSS) in anaerobic reactors. Biomass in aerobic tank was measured as mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS). Assays were performed according to Standard Methods for Examination of Water and Wastewater (APHA AWWA, 1992).

3.4.4 Amonium (NH4-N), Nitrite (NO_2-N), Nitrate (NO_3-N) and Phenol Measurements

Amonium (NH_4-N), nitrite (NO_2-N), nitrate (NO_3-N) and phenol were quantified using specific kits (Merck-Spectroquant), and spectrometric methods. The ammonia nitrogen, nitrite nitrogen, nitrate nitrogen, and phenol were measured in spectrophotometer (Nova 60-Merch) using Merck kits numbered 14752, 14547, 14773, and 14551, respectively.

3.4.5 Total Bicarbonate Alkalinity (Bic.Alk.) and Total Volatile Fatty Acid (TVFA) Measurements

Bicarbonate alkalinity (Bic.Alk.) and total volatile fatty acid (TVFA) concentrations were measured simultaneously using titrimetric method proposed by Anderson & Yang, (1992). The test was carried out as follows: firstly the pH of the sample was measured, secondly the sample was titrated with standard sulphuric acid (0.1 N) through two stages (first to pH=5.1, then from 5.1 to 3.5), and finally the VFA and Bic.Alk. concentrations were calculated with a computer program by solved the Eqs (3.1) and (3.2).

$$\mathrm{A}_1 = \frac{\left[HCO_3^-\right]*([\mathrm{H}]_2 - [\mathrm{H}]_1)}{[\mathrm{H}]_1 + \mathrm{K}_\mathrm{C}} + \frac{[VA]*([H]_2 - [\mathrm{H}]_1)}{[\mathrm{H}]_2 + \mathrm{K}_\mathrm{VA}} \tag{3.1}$$

$$\mathrm{A}_2 = \frac{\left[HCO_3^-\right]*([\mathrm{H}]_3 - [\mathrm{H}]_1)}{[\mathrm{H}]_3 + \mathrm{K}_\mathrm{C}} + \frac{[VA]*([H]_3 - [\mathrm{H}]_1)}{[\mathrm{H}]_3 + \mathrm{K}_\mathrm{VA}} \tag{3.2}$$

where A_1 and A_2 are the molar equivalent of the standard acid consumed to the first and second end points; $[HCO_3^-]$ the bicarbonate concentration; $[VA]$ the volatile fatty acid ion concentration; $[H]_{1,2,3}$ the hydrogen ion concentrations of the original sample and at the first and the second end points; K_C is the conditional dissociation constant of carbonic acid; K_{VA} is the combined dissociation constant of the volatile fatty acids (C_2–C_6), this pair of constants was assumed, being 6.6×10^{-7} for bicarbonate and 2.4×10^{-5} for volatile acids.

3.4.6 pH, Temperature, Dissolved Oxygen (DO) and Oxidation Reduction Potential (ORP) Measurements

The pH, temperature, and dissolved oxygen were measured using pH meter (WWT pH 330), an electronic digital heater and an oxygenmeter (WWT Oxi330/SET), respectively. The oxidation reduction potential was measured using Sen Tix ORP digital electrode (WWT pH 330) with an Ag/AgCl$_2$ reference electrode which is saturated with KCl solution and Pt electrode.

3.4.7 p-Nitrophenol (p-NP) and p-Aminophenol (p-AP) Measurements

p-NP was measured using Tris–HCl acid at a wavelength of 400 nm in an UV–vis spectrophotometer (Oren, Garevich, & Henis, 1991). Firstly, the samples were centrifuged at 4000 rpm for 10 minute. Then supernatant fluids were diluted fourfould with 1M tris hydrochloride. pH was adjusted to 9 before spectrophotometric measurement. The p-Nitrophenol (p-NP) was measured at a wave-length 400 nm with a Pharmacia LKBNovaPec II model spectrophotometer.

p-Aminophenol (p-AP) was determined by reaction with p-methyl aminobenzaldehyde. Firstly, the samples were centrifuged at 4000 rpm for 10 min. 0.8 ml of water and 0.05 ml of 1M HCl were added to 0.2 ml of supernatant. Then, 3 ml of ethanol, 0.5 ml of 5% p-dimethylmethylaminobenzaldehyde solved in ethanol and 0.5 ml of 15.7% citric acid in 6% NaOH were added. After 10 min., 2.5 ml of water was added and it was measured at a wavelength of 440 nm with a Pharmacia LKBNovaPec II model spectrophotometer (Oren, Garevich, & Henis, 1991).

3.4.8 Nitrobenzene (NB) and Aniline Measurements

Nitrobenzene (NB) and aniline measurements were carried out using a high-pressure liquid chromatography (HPLC) (Agilent-1100) with a method developed by EPA (EPA, 1994). Determined chromatographic conditions to monitor the nitrobenzene and aniline are given in Table 3.1.

Table 3.1 Chromatographic conditions for HPLC analyses in order to determine the nitrobenzene and aniline.

Column type	C-18 reverse phase HPLC column, 25-cm x 4.6-mm, 5 µm, (Ace 5C18)
Mobile Phase	50/50 (v/v) methanol/organic-free reagent water
Flow Rate	1.4 ml/min
Injection volume	10-µl
UV Detector	202 nm and 234 nm

Initially, all samples were centrifuged in centrifuge (SED 5X model) to remote any particulate matter and then filtered through a 0.45 µm pore sized teflon filter using disposable syringe (Agilent 5185-5835) prior to HPLC analysis. Elution prepared with isocratic solvent system consisting of 50% methanol and 50% organic-free reagent water. Thereafter it was run at a flow-rate of 1.4 ml/min. The autosampler was set for an injection volume of 10µl. The chromatographic separation of the sample was performed at 25 °C. Detection was performed at 202 nm wave-length for p-Nitrophenol and at 234 nm wave-length for p-Aminophenol using an UV detector.

3.4.8.1 Quantification of Nitrobenzene (NB) and Aniline

Quantification was carried out by integration of the peak area. Limit of detection (LOD) for NB and aniline were 0.000119±0.011 and 0.0009±0.0069 mg/l, respectively. Standard deviation values for eight replicate concentrations were computed and multiplied with Student's t value for 99 % confidence limits in minimum concentration over 5 days (Kuntasan, Karman, Wang, & Tuncel, 2005). $t_{0.99}$ is equal to 2.998 for n=8-1(7). The limits of quantification (LOQ) for NB and aniline were 0.0329 and 0.02094 mg/l, respectively.

3.4.9 Measurements of Intermediate Products

Nitrosobenzene and catechol measurements were carried out using a high-pressure liquid chromatography (HPLC) (Agilent-1100) with a method developed by

EPA (EPA 8300). Chromatographic conditions to HPLC analyses were given in Table 3.1.

After all samples were centrifuged in centrifuge (SED 5X model) and filtered through a 0.45 µm pore sized teflon filter using disposable syringe (Agilent 5185-5835) all samples given in HPLC. Elution consisted of 50% methanol and 50% organic-free reagent water. The flow-rate of solvent was adjusted 1.4 ml/min. The autosampler was set for an injection volume of 10µl. The chromatographic separation of the sample was performed at 25 °C. Detection was performed at 202 nm wave-length for nitrosobenzene and catechol.

2,6-bis(1,1-dimethylethy)-4-methyl, which is degradation product of p-NP, and hydroxlaminobenzene, which is degradation product of nitrobenzene, under anaerobic conditions and was investigated using GS/MS (ShimadzuQP 5050A) analysis. All samples were centrifuged in centrifuge (SED 5X model), supernatant in centrifuge is taken. 20 ml supernatant sample is extracted with 10 ml t-bütylmethyleter. The ether phase is taken and it is evapored. Remaining sediment in the vial is dissolved into methanol and is gived in GC-MS. Determined chromatographic conditions are given as follows:

Column type: Optima delta-3 0,25 µm 30metre*0,25 mm

Colum temperature: from 60 °C to 300 °C at 10 min.

Detector: 300 nm

3.4.10 Anaerobic Toxicity Assay (ATA) and Specific Methanogenic Activity (SMA)

ATA test was performed at 35°C using serum bottles with a capacity of 150 ml as described by Owen, Stuckey, Healy, Young, McCarty, (1979) and Donlon et al. (1996). Serum bottles were filled with 2000 mg VSS/l of biomass, 3000 mg /l of glucose-COD, suitable volume from the Vanderbilt mineral medium, 667 mg /l of sodiumthioglycollate providing the reductive conditions and 5000 mg /l of $NaHCO_3$ for maintaining the neutral pH. Before ATA test, the serum bottles were batch operated until the variation in daily gas production was less than 15% at least for 7 consecutive days. After observing the steady-state conditions, increasing concentration p-Nitrophenol and nitrobenzene were administered to serum bottles as slug-doses from concentrated stock solutions of these chemicals. The effects of p-NP and nitrobenzene on methane gas production were compared with the control samples. Inhibition was defined as a decrease in cumulative methane compared to the control sample. IC_{50} value indicates the 50% inhibition of methane gas production in serum bottles containing toxicant. This value shows the presence of toxicity. This value shows the toxicant concentration caused 50% inhibition in the methane gas production.

The SMA test was conducted in 150 ml serum bottles at 35 °C under anaerobic conditions. Serum bottles were filled with 3000 mg/l of glucose-COD, with suitable amount of Vanderbilt mineral medium, 667 mg/l of sodiumthioglycollate for to provide the reductive conditions and 5000 mg/l of $NaHCO_3$ for maintaining the neutral pH and 2000 mg VSS/l of biomass. Maximum specific methanogenic activity was calculated from the total methane production through 3 days with the method proposed by Owen et al., (1979) as follows:

$$\text{SMA (gCH}_4\text{-COD/g VSS day)} = \frac{\text{produced methane volume (ml) x 395 ml/ 1 gCOD}}{\text{sample (ml) x incubation time (day) x biomass concetration (g/l)}}$$

3.4.11 Toxicity Measurements

3.4.11.1 LUMIStox Toxicity Assay

A specific strain of the marine bacterium, Photobacterium phosphoreum, was used in this test to determine the toxicity of p-NP and NB. Reductions in light intensity at 5th, 10th and 30th min are chosen to measure the toxicity (Lange, 1994; EPS, 1992). The standard culture, Photobacterium phosphoreum (LCK480), was obtained from Dr.LANGE industrial measurement technique in Germany. Microtox testing was performed according to the standard procedure recommended by the manufacturer (Lange, 1994). The bioluminescense of the sample was measured in a luminometer (LUMIStox). Before toxicity assay, the pH of sample was adjusted between 5.5 and 8.5 using 0.1 N NaOH or HCI. Temperature of room should be between 15°C and 24 °C. Samples are serially diluted with 2% NaCl (w/v). The sodium chloride (2%) is used as the control. Samples containing bacterial luminescence were measured for 5, 15 and 30 min incubation times in a luminometer, respectively. The decrease in bioluminescence was indicated the toxic effect of the samples. Toxicity evaluation criteria for luminescent bacteria explained with the percent inhibition effect (H). Toxicity evaluation criteria for luminescent bacteria are presented in Table 3.2. If the percent inhibitory effect (H) change between 0% and 5%, the effect is non-toxic. When it is between 5% and 20%, the effect is possibly toxic, and when % inhibitor effect is between 20 and 90%, the effect is toxic (Lange, 1994).

Table 3.2 Effect of the samples on luminescent bacteria (Lange, 1994).

% inhibition (H)	**Effect**
0<H<5	Non toxic
5<H<20	Moderate toxic
20<H<90	toxic

3.4.11.2 Daphnia Magna Toxicity Test

Toxicity was tested using 24 h born *Daphnia magna* as described in Standard Methods (2005). Test animals were obtained from the Faculty of Water products in Aegean University in Izmir. After preparing the test solution, experiments were carried out using 5 or 10 daphnids introduced into test vessel. These vessels were controled with 100 ml of effective volume at 7-8 pH, providing minimum dissolved oxygen concentration of 6 mg/l at a ambient temperature of 20-25°C. Young *Daphnia magna* are used in the test (in first start ≤24 h old). A 24 h exposure is generally accepted for a *Daphnia* acute toxicity test. Results were expressed as mortality percentage of the *Daphnids.* The immobile animals which were not able to move were determined as the death of *Daphnids.*

3.4.12 Abiotic, Volotilization and Accumulation Tests of p-NP and NB

3.4.12.1 Abiotic Transformation Test

Nitrophenol and nitrobenzene measurement ware carried in the serum bottles containing autoclaved anaerobic granules of 1.2 gVSS/l after 25 days of incubation at 37°C. Abiotic test was consisted from control and test bottles. The control bottles no contained no nitrophenol and nitrobenzene. p-NP and NB concentrations in samples taken from the control and test bottles were measured periodically.

3.4.12.2 Volotilization Test

p-NP and NB concentrations were monitored in autoclaved anaerobic serum bottles containing no granules in order to determine the p-NP and NB transformation by volatilization at 35 °C. After an incubation time of 15 days, p-NP and NB concentrations in the serum bottles are measured.

3.4.12.3 Nitrophenol and Nitrobenzene Accumulation Inside Granular Sludge

A less amount sludge sample was withdrown from anaerobic AMBR and ABR reactor treating p-NP and NB. After the sludge samples were washed. The bottles was filled with distilled water and mixed by a magnetic stirrer (Hanna model) during three days in ambient conditions. After three days the p-NP and NB concentrations released to the water were measured in the supernatant. This test showed that whether p-NP and NB was accumulated into granular sludge.

3.4.13 Statistical Analysis

Anova analysis of varience between experimental date was performed to detect F and p values. In other words ANOVA test is used to test for differences amoung dependent and independent groups. The comparison between the actual variation of the experimental date averages and standard deviation is expressed in terms of F ratio. F is equal (found variation of the date averages/ expected variation of the date averages). p reports the significance level, d.f indicates the number of degrees of freedom.

Regression analysis was applied to the experimental date in order to determine the regression coefficient R^2. The aforementioned test was performed using Microsoft Excel program.

3.5 Operation Conditions

3.5.1 Start-up Period

The adaptation period is very important since the bacterial population used as seed is going to be exposed to the p-NP and NB in an anaerobic environment of the AMBR and ABR reactors. In order to acclimation the partially granulated biomass in the AMBR and ABR reactors, the anaerobic reactors were operated with synthetic wastewater through 45 days without p-NP and NB for reach to steady-state conditions. The steady state was arbitrarily considered as the variation of COD in the effluent and the variations of methane gas production and percentage less than 5% in concecutive 7 days. During the anaerobic phase the dissolved oxygen was zero and the redox potential was around -360 mV. During the aerobic phase the oxidation reduction potentials were between +90 and +80 mV. Start-up periods were 45 days for AMBR and 60 days for ABR.

3.5.2 Operation Parameters of Anaerobic Reactors (AMBR and ABR) and Aerobic Reactors

3.5.2.1 Sludge Retention Time (SRT, Θ_C)

Sludge retention time (SRT, θ_C) is the total quantity of active biomass in the reactor divided by the total quantity of active biomass withdrawn daily. Since no sludge wasting was applied for granule formation in the AMBR and ABR reactors, SRT in these reactors were determined using equations (3.3) and (3.4) (Metcalf & Eddy, 1991)

$$SRT = \frac{Vr \times Xr}{Qe \times Xe + Qw \times Xw} \qquad (3.3)$$

Q_w and X_w were defined as flow rate and microorganism concentrations, respectively in wasted sludge stream. The term Q_w*Xw only makes sense if there is a waste sludge stream. Since no sludge wasting was applied in the AMBR and ABR reactors, SRT can be expressed as follows:

$$SRT = \frac{Vr \times Xr}{Qe \times Xe} \tag{3.4}$$

The sludge wasting in a conventional CSTR reactor occurred from the settling tank and the solids in the effluent (X_e) were taken into consideration. Therefore, SRT in this reactor was calculated by using equation (3.6) with rearranged equation (3.5).

$$SRT = \frac{Vr \times Xr}{Qe \times Xe + Qw \times Xw} \tag{3.5}$$

V_r and X_r are effective volume of reactor and microorganism concentration in the aeration tank. Q_e and X_e were defined as flow rate and microorganism concentration measured in the settling tank. Q_W and X_W are the flow rate and microorganism concentration wasted from the reactor. The CSTRs used in this study are recycled reactors. In other words, the sludge was recycled 100% from the settling tank to the aeration tank. If the concentration of microorganism in the effluent of the settling tank is low, X_e is negligible (Metcalf & Eddy, 1991). In this study, the activated sludge was withdrawn from the inside of the aeration stage, the microorganism concentration in the reactor (X_r) was equal to the wasted microorganism concentration (X_w). Therefore, in this study the SRT in CSTR was calculated using equation (3.6).

$$SRT = \frac{V_r}{Q_w} \tag{3.6}$$

In this study, SRT (θc) in the CSTR reactor was adjusted as 20 days by discarding a certain amount of sludge volume from the aeration stage of the CSTR reactor. HRT in anaerobic reactors and CSTR were calculated using equation (3.7).

$$HRT = \frac{V_r}{Q} \tag{3.7}$$

V_r and Q were defined as reactor volume (l) and influent flowrate (l/day), respectively.

3.5.3 Operation Conditions for Sequential AMBR/CSTR Reactor System

Tables 3.3 and 3.4 show the operational conditions for AMBR and sequential AMBR/CSTR reactor system treating p-NP and NB. Firstly, the AMBR and CSTR reactors were operated through 140 days at HRTs of 10.38 days and 20 days, respectively, in order to investigate the effect of increasing p-NP concentrations on p-NP and COD removal performances, total and methane gas prouction, pH, TVFA, Bic.Alk. variations of sequential AMBR/CSTR reactor system. The influent COD concentration were kept constant at approximately 3000±100 mg/l corresponding to COD loading rate of 0.31 kg/m^{3}day through continuous operation. The p-NP concentration was increased subsequently from 10, 20, 30, 40, 50, 65, 85, to 100 mg/l corresponding to p-NP loading rates varied from 0.96 to 9.63 g/m^{3}day through continuous operation in AMBR reactor (see Table 3.3, Runs 1-8).

After this operation, the effect of HRT on p-NP and COD removal performances, total and methane gas production, pH, TVFA, Bic. Alk. variations was investigated in AMBR/CSTR system containing p-NP. The AMBR reactor was operated during 186 days with synthetic wastewater containing constant p-NP concentration of 40 mg/l and COD concentration of 3000±100 mg/l. HRT was decreased steeply from 10.38, 5.19, 3.4, 2.4, 1.5 to 1 day with increasing the organic loading rate from 0.31 to 3.25 kg/m^{3}day (see Table 3.3, Runs 9-14).

In another study performed with AMBR/CSTR reactor system the effect of increasing NB concentrations on treatment efficiencies of the AMBR/CSTR reactor system was investigated. The NB concentrations were increased steeply from 20, 40, 60, 100, 180, 250, 300 to 400 mg/l corresponding to the NB loading rates increasing from 0.96 to 9.63 g/m^{3}day through continuous operation of 131 days (see Table 3.4, Runs 1-8).

Finally, the effect of HRT on the performance of AMBR/CSTR reactor system was investigated in a synthetic wastewater containing 60 mg/l of NB. The influent COD and NB concentrations were kept constant at 3000±100 mg/l and 60 mg/l, respectively, during continuous operation of 94 days. These runs were performed at six different HRTs (See Table 3.4, Runs 9-14). The HRT was decreased from 10.38, 5.14, 3.5, 2, 1.5 to 1 day in AMBR. Therefore, HRT decreased from 6.92 to 0.67 day in aerobic CSTR reactor depending to flow rate entering to the CSTR. Total HRT was decreased from 17.3 to 1.67 day in sequential AMBR/CSTR reactor system. The AMBR and CSTR reactors were operated at steady state conditions approximately for 15-25 days in every increasing p-NP, NB concentrations and decreasing HRT.

Table 3.3 Operational conditions in AMBR and AMBR/CSTR reactor system for p-NP removal.

Runs	**Period (days)**	**HRT (day)**	**OLR**	**p-NP con.**	**NPLR**	**SRT (day)**	**Runs**	**Period (days)**	**HRT (day)**	**OLR**	**p-NP con.**	**NPLR**	**SRT (day)**
p-Nitrophenol removal													
Anaerobic (AMBR)							**Anaerobic (AMBR)/Aerobic (CSTR) reactor system**						
Run 1	15	10.38	0.31	10	0.96	250	**Run 1**	15	19.38	0.31	10	0.96	270
Run 2	17	10.38	0.31	20	1.92	265	**Run 2**	17	19.38	0.31	20	1.92	285
Run 3	18	10.38	0.31	30	2.89	280	**Run 3**	18	19.38	0.31	30	2.89	300
Run 4	18	10.38	0.31	40	3.85	348	**Run 4**	18	19.38	0.31	40	3.85	368
Run 5	17	10.38	0.31	50	4.81	352	**Run 5**	17	19.38	0.31	50	4.81	372
Run 6	19	10.38	0.31	65	6.26	350	**Run 6**	19	19.38	0.31	65	6.26	370
Run 7	16	10.38	0.31	85	8.19	360	**Run 7**	16	19.38	0.31	85	8.19	380
Run 8	20	10.38	0.31	100	9.63	380	**Run 8**	20	19.38	0.31	100	9.63	400
Run 9	35	10.38	0.31	40	3.85	340	**Run 9**	35	17.30	0.31	40	3.85	360
Run 10	33	5.19	0.6	40	7.71	280	**Run 10**	33	8.65	0.6	40	7.71	300
Run 11	27	3.4	0.93	40	11.76	180	**Run 11**	27	5.77	0.93	40	11.76	200
Run 12	34	2.4	1.31	40	16.67	151	**Run 12**	34	3.73	1.31	40	16.67	171
Run 13	32	1.5	2.14	40	26.67	125	**Run 13**	32	2.50	2.14	40	26.67	145
Run 14	25	1	3.25	40	40.00	110	**Run 14**	25	1.67	3.25	40	40.00	130

OLR= Organic loading rate (g COD/m^{3}day), HRT= Hydraulic retention time (day), SRT= Solid retention time (day), NPLR= p-NP loading rate (g p-NP/m^{3}day), p-NP con.= p-NP concentration (mg/l), (SRT= 20 day in aerobic CSTR reactor).

Table 3.4 Operational conditions of AMBR and AMBR/CSTR reactor system for NB removal.

Runs	**Period (days)**	**HRT (day)**	**OLR**	**NB con.**	**NBLR**	**SRT(day)**	**Runs**	**Period (days)**	**HRT (day)**	**OLR**	**NB.con**	**NBLR**	**SRT(day)**
Nitrobenzene removal													
Anaerobic (AMBR)							**Anaerobic (AMBR)/Aerobic (CSTR) reactor system**						
Run 1	18	10.38	0.31	20	1.93	620	**Run 1**	18	19.38	0.31	20	1.93	640
Run 2	18	10.38	0.31	40	3.85	635	**Run 2**	18	19.38	0.31	40	3.85	655
Run 3	19	10.38	0.31	60	5.78	643	**Run 3**	19	19.38	0.31	60	5.78	663
Run 4	13	10.38	0.31	100	9.63	620	**Run 4**	13	19.38	0.31	100	9.63	640
Run 5	15	10.38	0.31	180	17.34	645	**Run 5**	15	19.38	0.31	180	17.34	665
Run 6	17	10.38	0.31	250	24.08	670	**Run 6**	17	19.38	0.31	250	24.08	690
Run 7	14	10.38	0.31	300	28.90	680	**Run 7**	14	19.38	0.31	300	28.90	700
Run 8	17	10.38	0.31	400	38.54	740	**Run 8**	17	19.38	0.31	400	38.54	760
Run 9	15	10.38	0.31	60	5.78	757	**Run 9**	15	17.30	0.31	60	5.78	777
Run 10	16	5.19	0.6	60	11.56	384	**Run 10**	16	8.65	0.6	60	11.56	404
Run 11	15	3.4	0.93	60	17.14	238	**Run 11**	15	5.77	0.93	60	17.14	258
Run 12	16	2.4	1.31	60	30.00	198	**Run 12**	16	3.73	1.31	60	30.00	218
Run 13	16	1.5	2.14	60	40.00	103	**Run 13**	16	2.50	2.14	60	40.00	123
Run 14	16	1	3.25	60	60.00	65	**Run 14**	16	1.67	3.25	60	60.00	85

OLR= Organic loading rate (g COD/m^3day), HRT= Hydraulic retention time (day), SRT= Solid retention time (day), NBLR= NB loading rate (g NB/m^3day), NB con.= NB concentration (mg/l), (SRT= 20 day in aerobic CSTR reactor).

3.5.4 Operation Conditions for Sequential ABR/CSTR Reactor System

Tables 3.5 and 3.6 show the operational conditions for ABR and sequential ABR/CSTR reactor system treating p-NP and NB. Firstly, the ABR/CSTR reactor system was operated through 153 days in order to investigate the effect of increasing p-NP concentrations on the performance of reactor. p-NP concentrations increased steeply from 10 until 700 mg/l (see Table 3.5, Runs 1-12). This corresponds to p-NP loading rates of 0.96 and 67.9 g/m^3 day in ABR reactor, respectively. HRT was kept constant at 10.38 days during continuous operation in ABR reactor. The influent COD concentration and COD loading rate were kept constant at approximately 3000 ±100 mg/l and 0.289 g/m^3 day, respectively, through anaerobic operation. Secondly, the effect of HRT on the performance of ABR/CSTR system was investigated in a synthetic wastewater containing p-NP. The operation periods for HRT were given in Table 3.5 (See Runs 13-18). p-NP concentration was kept constant at 85 mg/l through continuous operation of 97 days. HRT decreased subsequently from 10.38, 5.19, 3.4, 2.4, 1.5 to 1 day with increasing the organic loading rates from 0.31 to 3.25 kg/m^{3}day in ABR reactor. Thirdly, the treatability performance of synthetic wastewater containing NB was investigated in ABR/CSTR reactor system. The ABR/CSTR reactor system was operated through 183 days at a constant HRT of 10.38 days and increasing NB concentrations (See Tale 3.6, Runs 1-10). NB concentrations were increased steeply from 20, 30, 55,130, 150, 210, 300, 450, 550 to 700 mg/l and the effect of increasing NB concentration on the COD and NB removal efficiencies, total and methane gas production, pH, TVFA, Bic.Alk. variations were investigated in sequential ABR/CSTR reactor system.

Finally, the effect of HRT on the performance of ABR/CSTR reactor system was investigated for synthetic wastewater containing 130 mg/l nitrobenzene. The ABR reactor was operated through 102 days at six HRTs (10.38, 5.14, 3.5, 2.0, 1.5 and 1 day) (See Tale 3.6, Runs 11-16). The ABR and CSTR reactors were operated at steady state conditions approximately for 15-25 days in every p-NP, NB concentration and HRT. The p-NP, NB concentrations and HRT were not decreased before reaching steady-state conditions.

Table 3.5 Operational conditions for ABR and ABR/CSTR reactor system for p-Nitrophenol removal.

Runs	**Period (days)**	**HRT (day)**	**OLR**	**p-NP con.**	**NPLR**	**SRT (day)**	**Runs**	**Period (days)**	**HRT (day)**	**OLR**	**p-NP con.**	**NPLR**	**SRT (day)**
p-Nitrophenol removal													
Anaerobic (ABR)							**Anaerobic (ABR)/Aerobic (CSTR) reactor system**						
Run 1	15	10.38	0.31	10	0.96	380	**Run 1**	15	19.38	0.31	10	0.96	400
Run 2	12	10.38	0.31	20	1.94	395	**Run 2**	12	19.38	0.31	20	1.93	415
Run 3	12	10.38	0.31	35	3.34	420	**Run 3**	12	19.38	0.31	35	3.37	440
Run 4	13	10.38	0.31	45	4.36	480	**Run 4**	13	19.38	0.31	45	4.34	500
Run 5	12	10.38	0.31	85	8.32	540	**Run 5**	12	19.38	0.31	85	8.19	560
Run 6	13	10.38	0.31	100	9.7	545	**Run 6**	13	19.38	0.31	100	9.63	565
Run 7	16	10.38	0.31	140	13.6	576	**Run 7**	16	19.38	0.31	140	13.49	596
Run 8	20	10.38	0.31	250	24.2	590	**Run 8**	20	19.38	0.31	250	24.08	610
Run 9	13	10.38	0.31	350	33.9	592	**Run 9**	13	19.38	0.31	350	33.72	612
Run 10	16	10.38	0.31	500	48.5	598	**Run 10**	16	19.38	0.31	500	48.17	618
Run 11	12	10.38	0.31	600	58.2	615	**Run 11**	12	19.38	0.31	600	57.80	635
Run 12	14	10.38	0.31	700	67.9	612	**Run 12**	14	19.38	0.31	700	67.44	632
Run 13	20	10.38	0.31	85	7.71	757	**Run 13**	20	13.59	0.31	80	7.71	777
Run 14	19	5.19	0.6	85	15.41	475	**Run 14**	19	6.83	0.31	80	15.41	495
Run 15	13	3.4	0.93	85	23.53	379	**Run 15**	13	4.46	0.31	80	23.53	399
Run 16	20	2.4	1.31	85	33.33	198	**Run 16**	20	3.15	0.31	80	33.33	218
Run 17	15	1.5	2.14	85	53.33	103	**Run 17**	15	1.97	0.31	80	53.33	123
Run 18	15	1	3.25	85	80.00	48	**Run 18**	15	1.31	0.31	80	80.00	68

OLR=Organic loading rate (g COD/m^3day), HRT= Hydraulic retention time (day), SRT= Solid retention time (day), NPLR=p-NP loading rate (g p-NP/m^3day), p-NP con.=p-NP concentration (mg/l), (SRT= 20 day in aerobic CSTR reactor).

Table 3.6 Operation conditions for ABR and ABR/CSTR reactor system for Nitrobenzene removal.

Runs	**Period (days)**	**HRT (day)**	**OLR**	**NB con.**	**NBLR**	**SRT (day)**	**Runs**	**Period (days)**	**HRT (day)**	**OLR**	**NB con.**	**NBLR**	**SRT (day)**
Nitrobenzene removal													
Anaerobic (ABR)							**Anaerobic (ABR)/Aerobic (CSTR) reactor system**						
Run 1	19	10.38	0.31	10	0.96	519	**Run 1**	19	19.38	0.31	10	0.96	539
Run 2	17	10.38	0.31	30	2.89	561	**Run 2**	17	19.38	0.31	30	2.89	581
Run 3	16	10.38	0.31	55	5.30	581	**Run 3**	16	19.38	0.31	55	5.30	601
Run 4	18	10.38	0.31	130	12.52	654	**Run 4**	18	19.38	0.31	135	13.01	674
Run 5	18	10.38	0.31	150	14.4	695	**Run 5**	18	19.38	0.31	173	16.67	715
Run 6	16	10.38	0.31	210	20.23	650	**Run 6**	16	19.38	0.31	210	20.23	670
Run 7	15	10.38	0.31	300	28.9	643	**Run 7**	15	19.38	0.31	300	28.9	663
Run 8	18	10.38	0.31	400	38.54	670	**Run 8**	18	19.38	0.31	400	38.54	690
Run 9	16	10.38	0.31	550	52.99	620	**Run 9**	16	19.38	0.31	550	52.99	640
Run 10	15	10.38	0.31	700	67.44	600	**Run 10**	15	19.38	0.31	700	67.44	620
Run 11	15	10.38	0.31	130	9.63	371	**Run 11**	15	13.59	0.31	100	9.63	391
Run 12	19	5.19	0.6	130	19.27	254	**Run 12**	19	6.83	0.31	100	19.27	274
Run 13	15	3.4	0.93	130	29.41	182	**Run 13**	15	4.46	0.31	100	29.41	202
Run 14	18	2.4	1.31	130	41.67	205	**Run 14**	18	3.15	0.31	100	41.67	225
Run 15	15	1.5	2.14	130	66.67	148	**Run 15**	15	1.97	0.31	100	66.67	168
Run 16	15	1	3.25	130	100.00	109	**Run 16**	15	1.31	0.31	100	100.00	129

OLR=Organic loading rate (g COD/m^{3}day), HRT= Hydraulic retention time (day), SRT= Solid retention time (day), NBLR=NB loading rate (g NB/m^{3}day), NB con.=NB concentration (mg/l), (SRT= 20 day in aerobic CSTR reactor).

3.6 Kinetic Approaches in Anaerobic Continuous Studies

Process modeling is a useful tool for the evaluation of the persistence of organic pollutants as well as to predict a bioreactor performance with respect to the degradation of organic compounds. Kinetic models are used to determine the importance of the relationships between variables to guide the experimental design and to evaluate the experimental results. These models also used to control and predict the treatment plant operation performance and to optimize the plant design and the results of scale-up pilot studies (Iza, Colleran, Paris, & Wu, 1991).

3.6.1 Application of Kinetic Model for AMBR and ABR Reactors

3.6.1.1 Substrate Removal Kinetics

3.6.1.1.1 Application of Monod Kinetic. For a completely mixing AMBR and ABR reactors with no biomass recycle, microbial and substrate mass balance can be expressed using Eq.3.8 and Eq.3.9.

Microbial Mass Balance

A microbial mass balance for the reactor can be described as follows:

$$\begin{bmatrix} microbial \\ change \\ rate \end{bmatrix} = \begin{bmatrix} microbial \\ input \\ rate \end{bmatrix} + \begin{bmatrix} microbial \\ growth \\ rate \end{bmatrix} - \begin{bmatrix} microbial \\ death \\ rate \end{bmatrix} - \begin{bmatrix} microbial \\ output \\ rate \end{bmatrix} \qquad (3.8)$$

Mathematically, Eq (3.8) can be written as Eq (3.9).

$$\frac{dx}{dt} = \frac{Q}{V} * X_i + m * X_r - k_d * X - \frac{Q}{V} * X_e \qquad (3.9)$$

where

V, Q , X_i , X_r , X_e are defined as the reactor volume (l), the flow rate (l/day), the concentration of biomass in the influent (g/l), the concentration of biomass in the reactor (g/l) and the concentration of biomass in the effluent (g/l). µ and k_d are specific growth rate (day^{-1}) and the endogenous decay coefficient (day^{-1}).

The concentration of biomass in the influent is very small and can be neglected ($X_i = 0$). Also, there is no change in the microbial mass at steady state conditions ($dX/dt = 0$). Therefore , Eq (3.9) can be written as Eq (3.10).

$$m - k_d = \frac{Q}{V} * \frac{X_e}{Xr} \tag{3.10}$$

Since no sludge wasting was applied in the anaerobic reactors, sludge retention time (SRT=θ_C) was calculated from the Eq (3.11) based on both MLVSS concentration into reactor and MLVSS concentration in the effluent of reactor.

$$q_c = \frac{V}{Q} * \frac{X_r}{Xe} \tag{3.11}$$

Equation (3.11) can be rearanged as follows:

$$m - k_d = \frac{1}{qc} \tag{3.12}$$

Where; (µ-k_d) is the net specific growth rate,day^{-1}. Equation (3.12) indicates that the net microbial growth decreases as the sludge retention time (SRT=θ_c) increases.

The relationship between the specific growth rate and the rate limiting substrate concentration can be expressed by the Monod equation (3.13):

$$m = \frac{m_{\max} * S}{K_s + S} \tag{3.13}$$

Eq (3.13) can be rearenged as follows.

$$\frac{m_{\max} * S_i}{K_s + S_i} = \frac{1}{q_c} + k_d \tag{3.14}$$

$$\frac{q_c}{1+q_c * k_d} = \frac{K_s}{m_{\max}} * \frac{1}{S_i} + \frac{1}{m_{\max}} \tag{3.15}$$

The value of maximum specific growth rate (μ_{max}) (day^{-1}) and half saturation concentration (K_s) (mg/l) could be determined by plotting the Eq (3.15). The value of μ_{max} can be calculated from the intercept of the straight line while K_S can be obtained from the slope of the line.

Substrate Mass Balance:

A substrate mass balance for the reactor can be described as Eq (3.169.

$$\begin{bmatrix} substrate \\ change \\ rate \end{bmatrix} = \begin{bmatrix} substrate \\ input \\ rate \end{bmatrix} - \begin{bmatrix} substrate \\ utilization \\ rate \end{bmatrix} - \begin{bmatrix} substrate \\ output \\ rate \end{bmatrix} \tag{3.16}$$

Mathematically, Eq (3.16) can be written as Eq (3.17).

$$\frac{dS}{dt} = \frac{Q}{V} * S_i - (m - k_d) * \frac{X_r}{Y} - \frac{Q}{V} * S_e \tag{3.17}$$

dS/dt is defined as the rate of substrate removal (g/l day). S_i and S_e are influent substrate concentration (g/l) and the effluent substrate concentration (g/l), respectively. *Y* is defined the growth yield coefficient (mass cell produced mass substrate utilized) (g VSS/g COD).

At steady rate *dS/dt* is 0. Thus, substrate balance at equilibrium can be rewritten as Eq (3.18).

$$\frac{(S_i - S_e)}{q_h} = (m - k_d) * \frac{X_r}{Y} \tag{3.18}$$

The equation given above can be reduced to equation (3.19)

$$\frac{(S_i - S_e)}{q_h} = \frac{X_r}{Y} * \left(\frac{1}{q_c} + k_d \right) \tag{3.19}$$

The kinetic parameters Y (g VSS / g COD), k_d can be obtained by rearranging Eq (3.19) as shown below:

$$\frac{(S_i - S_e)}{q_h * X_r} = \frac{1}{Y} * \left(\frac{1}{q_c} \right) + \frac{1}{Y} * k_d \tag{3.20}$$

The values of Y and k_d can determined by plotting $(1/\theta_c)$ versus $(S_i - S_e)/(Xr^* \theta_h)$. The value of k_d can be calculated from the intercept of the straight line while Y can be obtained from the slope of the line.

3.6.1.1.2 Contois kinetic model. The relationship between specific growth rate and limiting substrate concentrations was given as follows (Contois, 1959).

$$m = \frac{m_{\max} * S}{b * X_r + S} \tag{3.21}$$

where;

β is the contais kinetic parameter (g COD/g biomass).

By substituting Eq (3.21), instead of the Monod equation, into Eq (3.9) can be obtained Eq (3.22) can be obtained.

$$\frac{m_{\max} * S_i}{b * X + S_i} = \frac{1}{q_c} + k_d \tag{3.22}$$

If Eq (3.22) is rearranged, Eq (3.23) is obtained

$$\frac{q_c}{1 + q_c * k_d} = \frac{b}{m_{\max}} * \frac{X_r}{S_i} + \frac{1}{m_{\max}} \tag{3.23}$$

Similarly, the values of μ_{max} and β can be obtained by plotting the Eq (3.23). The value of μ_{max} can be calculated from the intercept of the straight line and finally, β can be obtained from the slope of the line.

*3.6.1.1.3 Grau First-Order Multicomponent Substrate Removal Model.*The general equation of a first-order kinetic model was illustrated in Eq (3.24) (Grau, Dohanyas, & Chudoba, 1975).

$$\frac{dS}{dt} = \frac{Q}{V} * S_i - (m - k_d) * \frac{X_r}{Y} - \frac{Q}{V} * S_e \tag{3.24}$$

The value of microorganism concentration in the reactor (X_r) (g/l) was constant during the experiment and, hence, $k_f^{'}$ $*X_r$ is a constant (k_f) ((mg/l day). If Eq. (3.23) is integrated and then linearilized, Eq (3.25) is obtained:

k_f’ and X_r are defined as first order multicompenant substrate removal kinetic constant (1/day) and the concentration of microorganism in reactor (gVSS /l), respectively.

$$-\ln\left(\frac{S_e}{S_i}\right) = \frac{k_f * q_h}{S_i} \tag{3.25}$$

$$-\ln\left(\frac{S_e}{S_i}\right) = p * q_h \tag{3.26}$$

p is equal to k_f /S_i (1/day) in order to calculate the constant of the first order model the equation (3.26) transformed to Eq 3.27. S_i and Se are defined as the initial organic matter concentration (g COD/l) and effluent organic matter concentration (g COD/l), repectively.

As p and S_i are constants for each loading conditions studied in the plot of –Ln (S_e/S_i) against θ_h (hydraulic retention time, day) will yield a straight line. By fitting the data to a linear function, using the least-squares method, the slopes (p) of the lines can be calculated. Finally k_f (mg/l day) can be determined by the Eq (3.27).

$$k_f = p * S_i \tag{3.27}$$

3.6.1.1.4 Grau Second-Order Multicomponent Substrate Removal Model. The general equation of a Grau second-order kinetic model is illustrated in Eq (3.28) (Grau, Dohanyas, & Chudoba, 1975, Öztürk, Altinbas, Arikan, & Demir, 1998).

$$-\frac{dS}{dt} = k_s * X_r * \left(\frac{S_e}{S_i}\right)^2 \qquad (3.28)$$

If Eq (3.28) is integrated and then linearilized, Eq (3.29) will be obtained:

$$\frac{S_i * q_h}{S_i - S} = q_h + \frac{S_i}{k_s * X} \qquad (3.29)$$

If the second term of the right part of Eq (3.29) is accepted as a constant, the Eq (3.30) will be obtained.

$$\frac{S_i * q_h}{S_i - S_e} = b * q_h + a \qquad (3.30)$$

k_S is second-order substrate removal rate constant (1/day). If Eq (3.29) re-arranged, Eq (3.30) will be obtained. This equation could be used to predict the effluent COD and p-NP concentrations.

$$S_e = S_i (1 - \frac{1}{(b + a / q_h)} \qquad (3.31)$$

$$N_e = N_i (1 - \frac{1}{(b + a / q_h)} \qquad (3.32)$$

Where;

a is equal S_i / (k_S *X) (day) and b are constant (dimensionless). (S_i-S_e)/S_e expresses the substrate removal efficiency and is symbolized as E (efficiency). S_e and S_i are effluent and influent COD concentrations (mg COD/l). N_e and N_i are effluent and influent p-NP concentrations (mg COD/l). X_r is the average biomass concentration in the reactor (mg VSS/l). θ_h is hydraulic retention time (day).

3.6.1.1.5 Zero Order Substrate Removal Model. The rate of change in substrate concentration in the system with assuming zero order model for substrate removal could be expressed in Eq (3.33) (Benefield, 1980)

$$-\frac{dS}{dt} = \frac{Q}{V} S_i - \frac{Q}{V} * S_e - k_0 \tag{3.33}$$

Under steady-state conditions, the rate of change in substrate concentration (-dS/dt) is negligible and the Eq (3.33) can be reduced to Eq (3.34) as follows: This equation could be used to predict the effluent COD and p-NP concentrations.

$$S_i - S_e = k_0 * q_h \tag{3.34}$$

where;

k_0 is zero order kinetic constant (mg/l.day) and it can be obtained from the slope of the line by plotting Eq (3.34).

3.6.1.1.6 First order substrate removal model. The rate of change in substrate concentration in the system with assuming the first order model for substrate removal could be expressed as follows (Benefield, 1980).

$$-\frac{dS}{dt} = \frac{Q}{V} S_i - \frac{Q}{V} * S_e - k_1 * S_e \tag{3.35}$$

Under steady-state conditions, the rate of change in substrate concentration (-dS/dt) is negligible and the equation given above can be reduced to the Eq (3.36):

$$\frac{S_i - S_e}{q_h} = k_1 * S_e \tag{3.36}$$

The value of first order kinetic constant (k_1) can be obtained by plotting S_i-S_e/θ_h versus S_e in Eq. (3.36). The value of k_1 can be obtained from the slope of the line. If Eq (3.36) re-arranged, Eq (3.37) will be obtained. This equation could be used to predict the effluent COD and p-NP concentrations. S_i and S_e are the substrate concentrations in the influent and effluent samples, respectively.

$$S_e = \frac{S_i}{k_1 \times Q_h + 1} \tag{3.37}$$

3.6.1.1.7 Second Order Substrate Removal Model. The rate of change in substrate concentration in the system with assuming the second order model for substrate removal could be expressed as follows (Benefield, 1980).

$$-\frac{dS}{dt} = \frac{Q}{V} S_i - \frac{Q}{V} * S_e - k_2 * S_e^{\ 2} \tag{3.38}$$

where;

k_2 is second order kinetic constant (l/mg.day)

Under steady-state conditions, the rate of change in substrate concentration (-dS/dt) is negligible and the equation given above can be reduced to Eq (3.39):

$$\frac{S_i - S_e}{q_h} = k_2 * S_e^{\ 2} \tag{3.39}$$

The value of k_2 can be obtained by plotting S_i-S_e/θ_h versus S_e^2 in Eq. (3.39). The value of k_2 can be obtained from the slope of the line.

3.6.1.1.8 Modified Stover-Kincannon Model. In this model, the substrate utilization rate is expressed as a function of the organic loading rate by monomolecular kinetic for biofilm reactors such as rotating biological contactors and biological filters. A special feature of Modified Stover-Kincannon model is the utilization of the concept of total organic loading rate as the major parameter to describe the kinetics of an anaerobic filter in terms of organic matter removal and methane production. A modified Stover-Kincannon model could be used for AMBR and ABR reactor as follows (Yu, Wilson, & Tay, 1998):

$$\frac{dS}{dt}=\frac{R_{\max}\times(Q\times S_i/V)}{K_B+(Q\times S_i/V)} \tag{3.40}$$

Where; d*S*/d*t* is defined in Eq. (3.41):

$$\frac{dS}{dt}=\frac{Q}{V}\times(S_i-S_e) \tag{3.41}$$

Eq (3.42) obtained from the linearization of Eq (3.41) as follows:

$$\frac{V}{Q\times(S_i-S_e)}=\frac{K_B}{R_{\max}}\frac{V}{Q\times S_i}+\frac{1}{R_{\max}} \tag{3.42}$$

If the maximum utilization rate (R_{max}) (g/l.day) and the saturation value constant (K_B) (g/l.day) values obtained for COD and p-NP were substituted in Eq (3.43), Eq (3.44) and (3.45) could be used to predict the effluent COD and p-NP concentrations, respectively. (QS_i/V) explain the organic loading rate (OLR) applied to the reactor. Q and V are the inflow rate (l/day) and the volume of the anaerobic reactor (l), respectively.

$$\frac{Q(Si-Se)}{V}=\frac{R_{\max}(QSi/V)}{K_B+(QSi/V)} \tag{3.43}$$

$$Se=Si-\frac{R_{\max}Si}{K_B+(QSi/V)} \tag{3.44}$$

$$Ne=Ni-\frac{R_{\max}Ni}{K_B+(QNi/V)} \tag{3.45}$$

3.6.1.2 Gas Production Kinetics

3.6.1.2.1 Modified Stover-Kincannon Gas Kinetic Model. The total gas production rate and methane quality can be mathematically explained in terms of substrate removal. The gas production and quality are dependent on the substrate removal and substrate loading rate. The model developed by Stover. Eq (3.46) was arranged and has been applied to determine the total and methane gas productions (Satyanarayan & Kaul, 2002).

$$G = \frac{G_{\max} \times (Q \times S_i / A)}{G_B + (Q \times S_i / A)} \tag{3.46}$$

where, A represents the total disc surface area whereby total biomass concentration immobilized on discs. The simple modification of the original Stover–Kincannon model is the introduction of total organic loading rate, QS_i/V into the Eq. (3.45) instead of QS_i/A, resulting in Eq (3.47).

$$G = \frac{G_{\max} \times (Q \times S_i / V)}{G_B + (Q \times S_i / V)} \tag{3.47}$$

Where, $(Q \times S_i / V)$ can be expressed as organic loading rate (OLR).This equation could be showed as follows for the total specific gas production rate: G is the specific gas production rate (ml/l.day) and G_{max} is defined as the maximum specific gas production rate (ml/l day). G_B is the proportionality constant (mg/l.day).

$$G = \frac{G_{\max} \times (OLR)}{G_B + (OLR)} \tag{3.48}$$

Eq 3.48 gives the total specific gas production rate. The specific methane gas production rate could also be explained with Eq (3.49). Where, M, M_{max} and M_C can be explained as specific methane gas production rate (ml/l.day), maximum specific methane gas production rate (ml/l.day) and proportionality constant (mg/l.day).

$$M = \frac{M_{\max} \times (OLR)}{M_B + (OLR)} \tag{3.49}$$

Where, M, M_{max} and M_B can be explained as the specific methane gas production rate (ml/l.day), the maximum specific methane gas production rate (ml/l.day) and the proportionality constant (mg/l.day) for methane production, respectively.

Linearization of Eqs (3.48) and (3.49) gives Eqs (3.50) and (3.51) which these equations could be used to determine the kinetic constants for specific total gas and methane gas productions:

$$\frac{1}{G} = \frac{G_B}{G_{\max}} \times \frac{1}{OLR} + \frac{1}{G_{\max}} \tag{3.50}$$

$$\frac{1}{M} = \frac{M_B}{M_{\max}} \times \frac{1}{OLR} + \frac{1}{M_{\max}} \tag{3.51}$$

*3.6.1.2.2 Van Der Meer and Heertjes Equation.*To describe the kinetic of a gas production, the following empirical equation (3.52) was used (Van der Meer & Heertjes, 1983).

$$V=k_{sg}\, Q\, (S_i-S_e) \tag{3.52}$$

In this model the methane production is related with Van der Meer and Heertjes kinetic constant (k_{sg}) (ml/mg), with flowrate applied to AMBR and ABR and removal efficiency of substrate. Where, V is methane production (ml/day), Q is wastewater flow rate (l/day). S_i and Se are explained as the influent substrate concentration (mg/l) and the effluent substrate concentration (mg/l), respectively.

3.6.1.2.3 Chen and Hasminoto Model. The ultimate methane yield (B_{max}) is defined as liter of the methane produced per gram of volatile solids added as the hydraulic retention time reaches infinity (Chen & Hasminoto, 1980). Ultimate methane yield (B_{max}) is usually determined by plotting the actual methane yield (B) versus $1/\theta_h$ and extrapolating the curve to $1/\theta_h$ =0. The biodegradable COD in the reactor will be directly proportional to (B_{max}-B), and B_{max} will be directly proportional to the biodegradable COD loading (Chen & Hashimoto, 1980).

Chen and Hasminoto model is expressed as follows;

$$\frac{S_i}{S_e} = \frac{k}{q_h \times m_{\max} + k - 1} \tag{3.53}$$

Substrate removal is an indicator of biogas production in anaerobic process as described in Eq (3.54). k is Chen and Hasminoto kinetic constant (dimensionless).

$$\frac{S_i}{S_e} = \frac{B_{\max} - B}{B_{\max}} \tag{3.54}$$

If the Eq (3.54) is linearized Eq (3.55) was obtained in terms of biogas yield (B).

$$B = B_{\max}(1 - \frac{k}{q_h \times m_{\max} + k - 1}) \tag{3.55}$$

If the Eq (3.55) is rearranged Eq (3.56) was obtained, the biogas yield (B) and the maximum specific growth rate (μ_{max}) (day^{-1}) of the methanogenic microorganisms can be calculated using Eq (3.56).

$$\frac{B_{\max}}{B_{\max} - B} = \frac{q_h \times m_{\max}}{k} + 1 - \frac{1}{k} \tag{3.56}$$

CHAPTER FOUR
RESULTS AND DISCUSSIONS

4.1 Batch Studies

4.1.1 Anaerobic Toxicity Assay (ATA) Results for p-NP and NB

The p-NP and NB concentrations caused 50% decreases in the methanogenic activity (decrease of methane gas production) were calculated as IC_{50} value. The IC_{50} value for p-NP and NB were found to be 27.7 mg/l and 109 mg/l, respectively as shown in Figures 4.1 and 4.2 Razo-Flores et al, (1997) reported that IC_{50} values of p-NP and NB were 0.061 mM (8.49 mg/l) and 0.081 mM (9.97 mg/l), respectively. In another study, the IC_{50} values for p-NP and NB were found as 4 mg/l and 13 mg/l, respectively (Speece, 1996). In our study, the IC_{50} values of p-NP and NB were higher than IC_{50} values reported by Razo-Flores et al., (1997) and Speece (1996). This could be attributed to the resistance of partially granulated sludge to p-NP and NB. According to IC_{50} values it can be concluded that p-NP exhibited higher toxicity compared to NB.

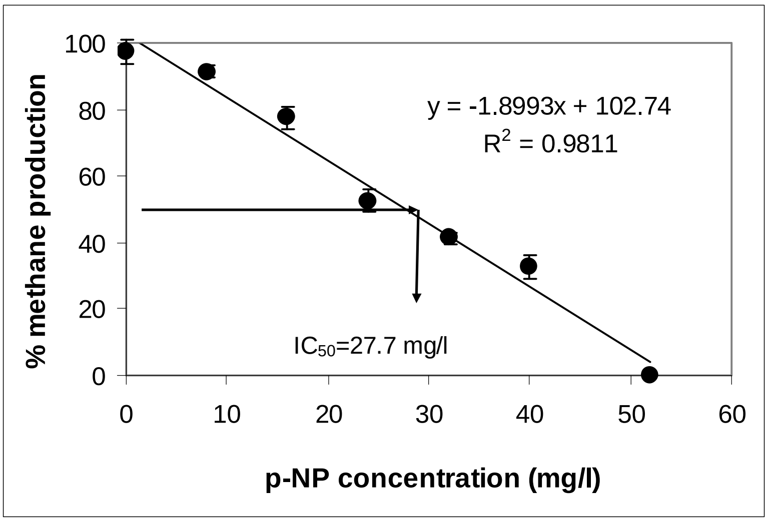


Figure 4.1 IC_{50} value for p-NP (IC_{50}= 27.7 mg/l).

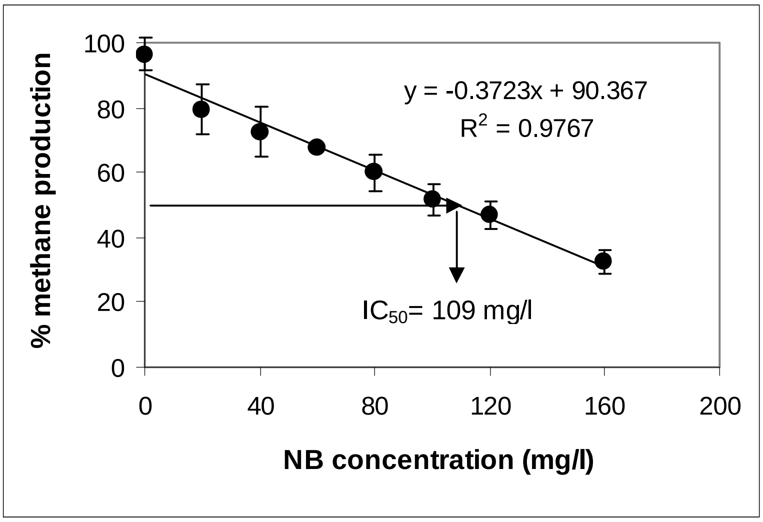


Figure 4.2 IC_{50} value for NB (IC_{50}= 109 mg/l).

4.1.2 Abiotic, Volotilization and Accumulation Test Results for p-NP and NB

4.1.2.1 Abiotic, Volotilization and Accumulation Test Results for p-NP

The abiotic test results were given in Table 4.1(a). 1.9 % of p-NP was adsorbed by anaerobic biomass (1.2 gVSS/l) through anaerobic incubation of 12 days. The abiotic transformation rate in abiotic experiment was found as 0.000125 mg p-NP/mg VSS day after 12 day and 0.00009 mg p-NP/mg VSS day after 25 day, respectively. These results were similar with the results reported by Uberoi & Bhattacharya, (1997). Uberoi & Bhattacharya, (1997) found that the abiotic transformation rate was 0.0004 mg NP/mgVSS day after 80 hours for p-NP. Therefore the abiotic p-NP removal was not taken into consideration.

40.5 mg/l of p-NP concentration was used at the beginning of the volatilization study in serum bottles. After 25 days of incubation time, 40.5 mg/l p-NP decreased to 39.9 mg/l. The results indicated that 1.5 % of p-NP was removed by volatilization after 15 days of anaerobic incubation (See Table 4.1(b)). Therefore it was concluded that p-NP removal with volatilization is not significant. In order to determine whether the p-NP is accumulated inside the granules, granules were mixed in distilled water for 3 days. The p-NP doses measured in the supernatant showed that 0.6 mg/l of p-NP is released to the water. As a result it can be said that 0.06 % of p-NP was accumulated into granular sludge (See Table 4.1(c)). This is not significant for p-NP removal.

Table 4.1 Batch abiotic study for p-NP (a).

Days	**0**	**5**	**12**	**Abiotic removal efficiency (%)**	**Abiotic transformation rate (mg p-NP/mgVSS day)**
Sterilized culture control (mg/l)	0	0	0	0	0
Culture with p-NP (mg/l)	42.5	42.1	41.7	1.9	0.00009-0.000125
Culture without p-NP (mg/l)	0	-	0	0	0

Table 4.1 Volatilization test for p-NP (b).

Days	**0**	**5**	**15**	**Volatilization losses (%)**
Sterilized vials (mg/l)	0	0	0	0
Vials with p-NP (mg/l)	40.5	39.9	39.9	1.5

Table 4.1 Volatilization and accumulation test for p-NP (c).

Days	**0**	**3**	**p-NP accumulation into granular sludge (%)**
The p-NP doses measured in the supernatant (mg/l)	0	0.6	0.06

4.1.2.2 Abiotic, Volotilization and Accumulation Test Results for NB

The abiotic test results were given in Table 4.2 (a). The test results for abiotic study showed that 1.2 % of NB was adsorbed by anaerobic biomass (500 VSS mg/l) through anaerobic incubation of 25 days. The initial NB concentration was 80 mg/l. After 25 day, NB concentration decreased to 79.0 mg/l. The abiotic transformation rate was found as 0.00013 mg NB/mg VSS day after 25 day. The results were similar compared with p-NP. Therefore abiotic removal does no an important removal mechanisms. For volatilization study the influent concentration of nitrobenzene was kept around 100 mg/l. Test bottles were incubated at 38°C through 15 days. After 10 days, the concentration of nitrobenzene in bottles was found to be around 98.7 mg/l. The results of test showed that the removal of NB by volatilization is 1.3% compared to influent NB concentration (see Table 4.2 (b)). This is not significant for NB removal. The NB doses measured in the supernatant showed that 0.4 mg/l of NB is released to the water. The result showed that 0.04 % of NB was accumulated into granular sludge, which is not significant for NB removal (see Table 4.2 (c)).

Table 4.2 Batch abiotic study for NB (a).

Incubation time (days)	**0**	**5**	**12**	**Abiotic removal efficiency (%)**	**Abiotic transformation rate (mg p-NP/mgVSS day)**
Sterilized culture control (mg/l)	0	0	0	0	0
Culture with NB (mg/l)	80	79.6	79.0	1.2	0.00013
Culture without NB (mg/l)	0	0	0	0	0

Table 4.2 Volatilization test for NB (b).

Incubation time (days)	**0**	**5**	**15**	**Volatilization losses (%)**
Sterilized vials (mg/l)	0	0	0	0
Vials with NB (mg/l)	100	98.7	98.7	1.3

Table 4.2 Volatilization and accumulation test for NB (c).

Incubation time (days)	**0**	**3**	**Accumulated into granular sludge (%)**
The NB doses measured in the supernatant (mg/l)	0	0.4	0.04

4.2 Continuous Studies

4.2.1 The Removal of P-NP in AMBR and Sequential AMBR/CSTR Reactor System

4.2.1.1 Start-up Period

The adaptation period is very important since the bacterial population used as seed is going to be exposed to the anaerobic environment of the AMBR reactor. To aclimated the partially granulated biomass in the AMBR reactor, this reactor was operated through 45 days without p-NP under steady-state conditions. The steady-state conditions were defined with COD removal efficiencies and methane gas productions higher than 90% and 45%, respectively, for concecuotive 5-7 days. During the anaerobic phase zero dissolved oxygen was observed and the redox potential was around -360 mV. During the aerobic phase the oxidation–reduction potential was between +80 and +90 mV. COD and methane gas variations through start-up period are shown in Figures 4.3 and 4.4, respectively. As shown in Figures 4.3 and 4.4, AMBR reactor reached steady-state conditions after an operation period of 30 days. After this operation time, COD removal efficiency and methane gas percentage remained constant approximately 93% and 52%, respectively, through continuous operation.

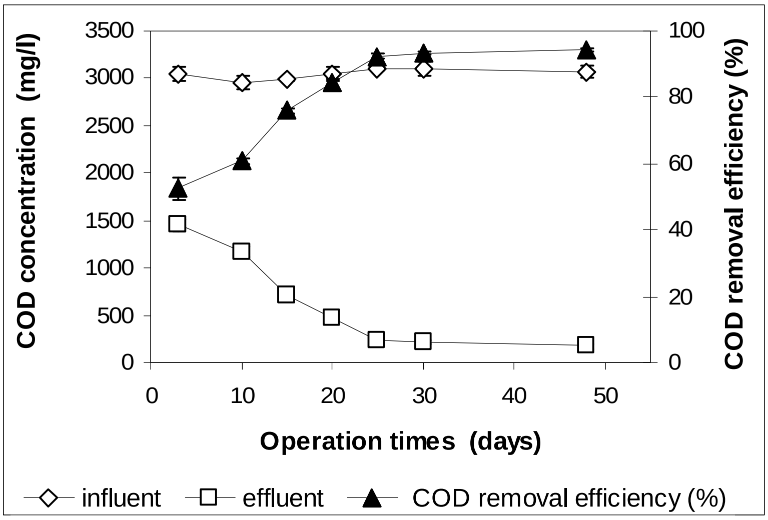


Figure 4.3 COD variations through start-up period in AMBR.

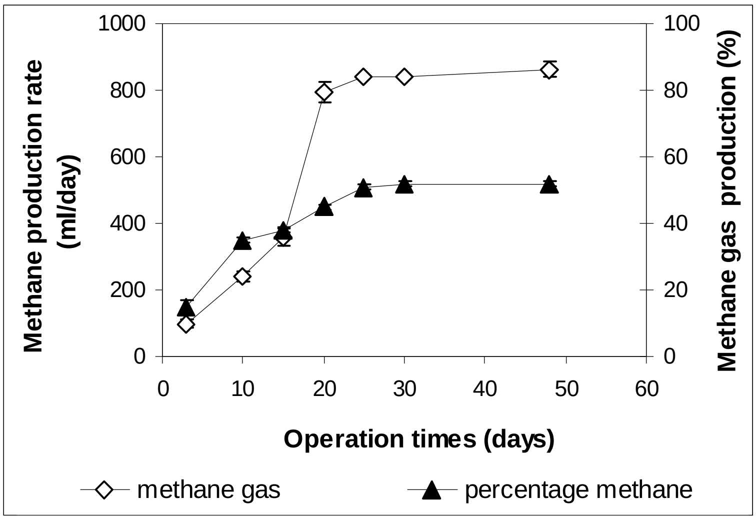


Figure 4.4 Methane gas variations through start-up period in AMBR.

4.2.1.2 Effect of Increasing p-Nitrophenol (p-NP) Concentration on Performance of AMBR Reactor

4.2.1.2.1 Effect of Increasing p-Nitrophenol Concentration on The COD Removal Efficiencies in AMBR Reactor. In this step of this study, the effect of increasing p-NP concentrations on COD removal efficiencies was investigated. The operation of the AMBR with p-NP was started at an influent p-NP concentration of 10 mg/l and a p-NP loading rate of 0.96 g/m^{3}day. Then the p-NP concentration was subsequently increased from 15, 20, 30, 35, 40, 50, 65, 85 to 100 mg/l corresponding p-NP loading rates of 1.53, 1.91, 2.89, 3.27, 3.85, 4.81, 6.26, 8.18 and 9.63 g/m^{3}day. The variations of COD with increasing p-NP loading rates are shown in Figure 4.5. As shown in this figure, the COD removal efficiency remained approximately 92 % until a p-NP loading rate of 3.85 g/m^{3}day corresponding a p-NP concentration of 40 mg/l. The effluent COD concentrations also were approximately 225 mg/l until a p-NP loading rate of 3.85 g/m^{3}day. After this p-NP concentration, COD removal efficiency decreased rapidly from 87% to 56%. The effluent COD concentration and removal efficiency were measured as 1414 mg/l and 56%, respectively, at a maximum p-NP loading rate of 9.63 g/m^{3}day. The optimum p-NP loading rate and p-NP concentration were found as 3.85 g/m^{3}day and 40 mg/l, respectively, for maximum COD removal efficiency of 92%.

In this study, 93% removal of COD was obtained at a p-NP loading rate of 3.85 g/m^{3}day corresponding a p-NP concentration of 40 mg/l. These removal rates are much higher than 90% COD and p-NP removal efficiencies in the treatment of a synthetic wastewater (glucose, and beef extract) being loaded with 900 mg p-NP/l day in an anaerobic biological fluidized-bed reactor system as reported by Tseng & Yang, (1994).

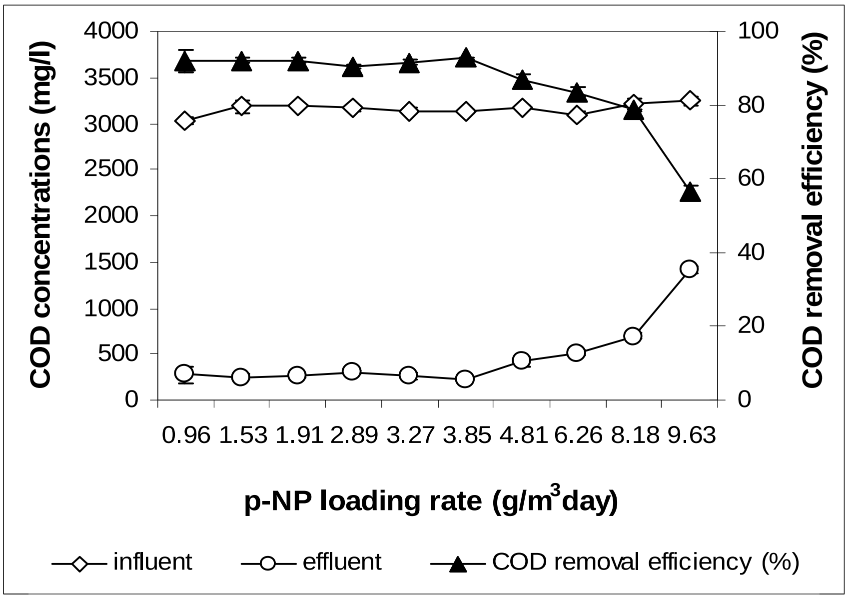


Figure 4.5 Effect of p-NP loading rate on COD removal efficiencies in AMBR.

4.2.1.2.2 Effect of p-Nitrophenol Loading Rate on the para-Nitrophenol (p-NP) Removal Efficiencies in AMBR Reactor. The effect of para-nitrophenol (p-NP) loading rate on the p-NP removal efficiencies is shown in Fig. 4.6. A p-NP removal efficiency of 85% was obtained at an initial p-NP concentration of 10 mg/l and at a p-NP loading rate of 0.96 g/m^{3}day. When the p-NP loading rate was increased from 0.96 to 3.85 g/m^{3}day the p-NP removal efficiency remained stable between 92% and 93%. After a p-NP loading rate of 3.85 g/m^{3}day, a slightly decrease in the p-NP removal efficiency was showed. The p-NP removal efficiency was around 91%. p-NP removal efficiency was found above 90% at all p-NP loading rates. The effluent p-NP concentration was measured as 9.6 mg/l (E=91%) at a maximum p-NP loading rate of 9.13 g/m^{3}day. 93% p-NP removal efficiency is much higher than that obtained by Haghighi-Podeh, Bhattacharya, & Qu, (1995) in a lab-scale digestor.

82% removal of p-NP was obtained at a OLR of 0.36 mg/l.day. Tseng & Yang, (1994) obtained a p-NP removal efficiency of 90% at p-NP loading rate of 900 mg/l.day in an anaerobic biological fluidized-bed reactor system.

As seen in Fig 4.5, the COD removal efficiency decreased from 92% to 52% as p-NP loading rate increased from 0.96 to 9.63 g/m^{3}day, while no variation was observed in p-NP removal (E=91-93%) (See Fig. 4.6). This result can be explained by the complete transformation of the p-NP to p-AP under reductive anaerobic conditions. However the breakdown products such as p-AP and phenol were not ultimately metabolized and caused residual COD. This caused rising of COD in the effluent of AMBR.

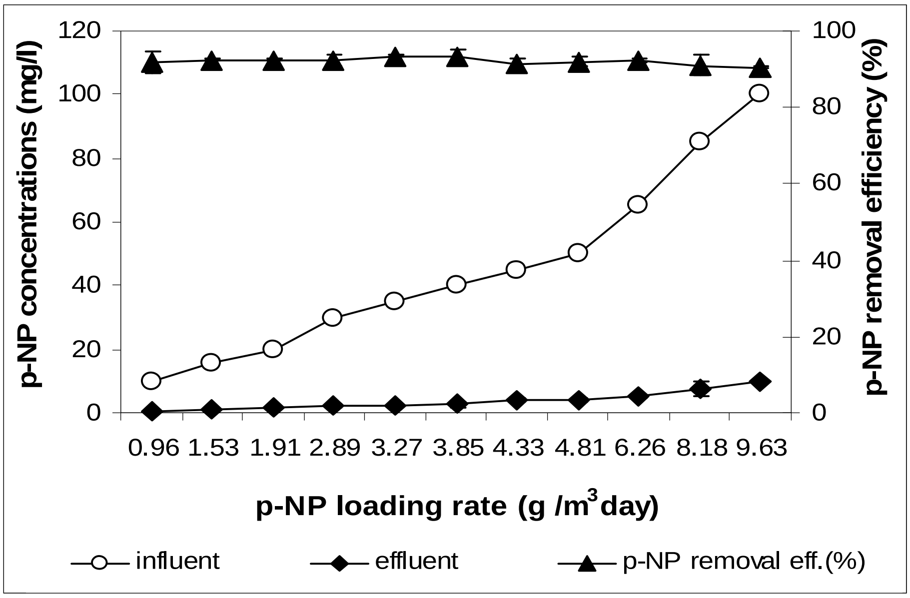


Figure 4.6 The effect of p-NP loading rate on p-NP removal efficiencies in AMBR.

4.2.1.2.3 Effect of p-Nitrophenol Loading Rate on the Total and the Methane Gas Production in AMBR Reactor. Figure 4.7 shows the effect of p-NP loading rate on the gas productions and methane gas percentage. The daily total gas, methane gas productions and methane percentage were recorded as 1450 ml/day and 850 ml/day and 52%, respectively at a p-NP loading rate of 0.96 g/m^{3}day. The maximum total gas, methane gas productions and methane percentage were found about 2300 ml/day, 1300 ml/day and 56%, respectively at a p-NP loading rate of 4.81 g/m^{3}day.

After this loading rate, methane percentage decreased rapidly from 56% to 43%. This indicated an inhibition effect of p-NP on methane *Archaea* at p-NP loading rates as high as 4.81 g/m^{3}day. p-NP loading rates varied between 0.96 g/m^{3}day and 9.63 g/m^{3}day could be used as carbon source by the methanogenic granular sludge resulting in high methane productions based on high COD removal under anaerobic conditions.

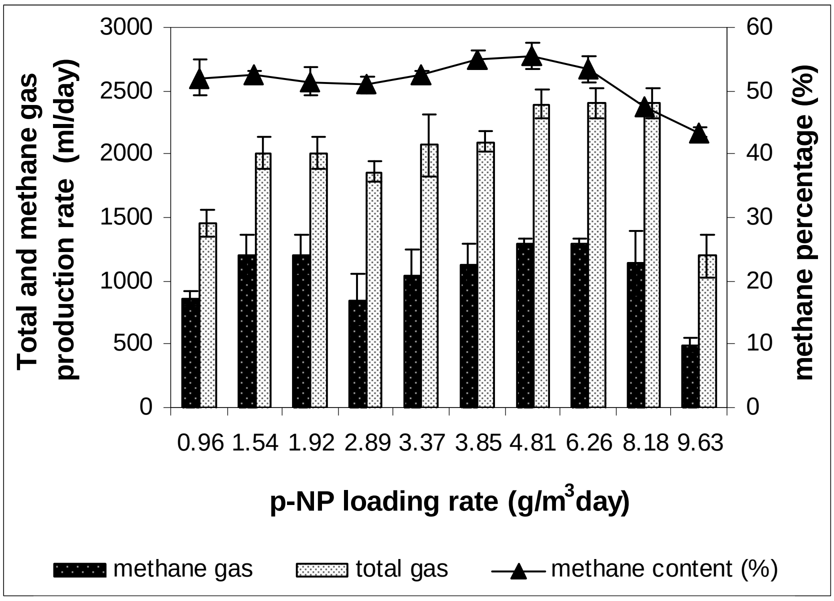


Figure 4.7 The effect of p-NP loading rate on total, methane gas production and methane percentage in AMBR.

4.2.1.2.3 Determine of Transformation Products of p-NP Under Anaerobic Conditions. Gorontzy, Kuver, & Botevogel, (1993) reported that nitrophenols are transformed partially or completely to its amino derivatives under methanogenic and sulphate reducing conditions. p-NP is transformed to p-AP under anaerobic conditions, which is its corresponding amine groups (Donlon et al., 1996; Karim & Gupta, 2001; Kuscu & Sponza, 2005; Melgoza & Buitron, 2001). Melgoza & Buitron, (2001) reported that p-NP is transformed completely to p-AP. In this study, p-NP was transformed to p-AP with efficiency near to 100% in anaerobic phase. p-AP did not show a significant transformation under anaerobic conditions. But some reports indicated that p-AP is mineralized by anaerobic bacteria (O’Conner & Young, 1996; Spain, Wyss, & Gibson, 1979, Tseng & Lin, 1994). The p-AP breaks down to phenol via deamination under methanogenic conditions as reported by

Tseng & Lin, (1994). Spain, Wyss, & Gibson, (1979) identified an enzyme namely 2-nitrophenol oxygenase from *Pseudomonas*, capable of removing the nitrite group from p-NP, and converting them to catechol.

In our study, p-AP and phenol observed in compartments and in the effluent of anaerobic reactors as the intermediate metabolites. This showed that p-NP is transformed to p-AP and then p-AP converted phenol under anaerobic conditions (Spain, Wyss, & Gibson, 1979; Tseng & Lin, 1994). In our study, catechol was no detected in compartment and the effluent of anaerobic reactor. The results of this study confirmed the findings of Karim & Gupta (2001). Tseng & Yang, (1994) investigated the metabolic pathway of nitrophenols in the anaerobic digester. The results indicated a high concentration of 2,6-bis(1,1-dimethylethy)-4-methyl phenol present in orto-NP samples except for the para-NP and meta-NP samples. In our study, we investigated whether 2,6-bis(1,1-dimethylethy)-4-methyl phenol present in the compartments and in the effluent of the anaerobic reactor using GS/MS analysis. Results showed that 2,6-bis(1,1-dimethylethy)-4-methyl phenol could not be found in samples taken from the compartment and from the effluent of anaerobic reactor, confirmed the findings of Tseng & Yang, (1994).

4.2.1.2.4 Effect of p-Nitrophenol (p-NP) Loading Rate on para-Aminophenol (p-AP) Productions. Studies on anaerobic biodegradation of p-NP showed that p-NP reduced to para-amino phenol (p-AP) as intermediate products under anaerobic conditions (Tseng & Lin, 1994). Figure 4.8 indicates the p-AP production through increasing p-NP loading rates in AMBR reactor. The production of p-AP was measured as 3.5 mg/l at an initial p-NP concentration of 10 mg/l. As shown in Fig. 4.8, when the p-NP loading rate was raised, the p-AP concentration also increased. For instance, the p-AP in the effluent was found to be 8 mg/l at a p-NP loading rate of 2.89 g/m^{3}day while the p-AP concentration was measured as 29 mg/l at a p-NP loading rate of 9.13 g/m^{3}day. Approximately 95 % of p-NP was transformed to p-AP until a p-NP loading rate of 3.85 g/m^{3}day. p-AP was less toxic compared p-NP (Karim & Gupta, 2001). On the basis of these data it can be concluded that the anaerobic detoxification mechanism was possible through the successfully operation of continuous fed laboratory scale AMBR reactors for the treatment of the highly toxic nitrophenolic compounds such as p-NP.

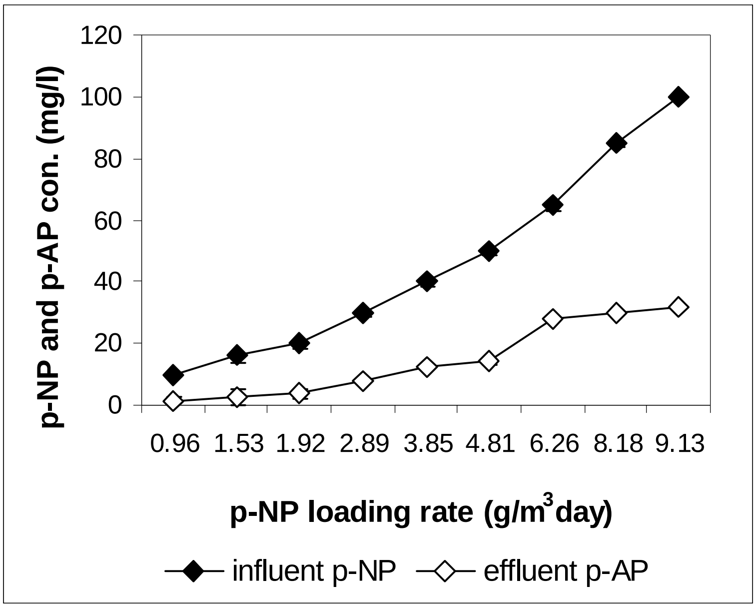


Figure 4.8 Effect of p-NP loading rate on p-aminophenol (p-AP) production in AMBR.

4.2.1.2.5 COD, p-NP Removal Efficiencies and p-AP Variations in Compartments of the AMBR and Phenol Variation in the Effluent of the AMBR Reactor at Increasing p-NP Loading Rates. Figure 4.9 shows the COD, p-NP removal efficiencies and p-AP variations in compartments of AMBR. As shown in Fig.4.9 (a) and (b), the COD and p-NP removal efficiencies were higher in the first compartment than the other two compartments. The COD and p-NP were removed approximately with efficiencies of 80% and 90%, respectively, in the initial compartment. Removal efficiencies of COD and p-NP in subsequent compartments were about 15-20% in the second compartment and 10-15% in the third compartment. Similarly the p-AP production was found to be higher in the initial compartment than the other two compartments. The COD and p-NP concentrations reduced from 3210 to 1580 mg/l and from 100 mg/l to 6.9 mg/l, respectively in the first compartment at an OLR of 9.63 g/m^{3}day. The COD and p-NP removal efficiencies were 51% and 88% in the first compartment, 23% and 5% in the second compartment and 12% and 5% in the third compartment at the aforementioned loading rate, respectively. Approximately 76–93% of the p-NP was converted to p-AP in initial compartment. Figure 4.9(c) shows the p-AP concentration in the compartment of AMBR at different p-NP loading rates. As shown in this figure, p-AP concentration in compartments increased as p-NP loading rate increased. This can be explained by the partially degradated of p-NP to p-AP under anaerobic conditions. p-AP concentration was higher in the first compartment than the other compartments at all p-NP loading rates. Then p-AP concentration decreased in compartment 2 and 3. For example the p-AP concentration decreased from 62 mg/l in compartment to 1.58 mg/l in the compartment 2; 40 mg/l in compartment 3, and to 38 mg/l in the effluent. Karim & Gupta, (2001) reported 4.44 mg/l p-AP in the effluent of upflow anaerobic sludge blanked reactor (UASB) feeding with 30 mg/l of p-NP at a HRT of 24 h. In our study, p-AP concentration in the effluent of AMBR was found as 9 mg/l at 30 mg/l of p-NP concentration corresponding to p-NP loading rate of 2.89 g/m^{3}day. The decradation of p-AP in compartments 2 and 3 showed that p-AP was degraded to another intermediate product under anaerobic conditions. Furthermore; p-AP concentrations observed in the effluent were lower than their stoichiometrically reduction values at all p-NP concentration introduced to the AMBR. For example stoichiometrically 30 mg/l of p-NP was gegraded to 23.53 mg/l of p-AP. This analys show that the p-NP was degraded to p-AP and p-AP converted to phenol. Figure 4.9(d) shows the phenol concentration in the effluent of AMBR at different p-NP loading rates. As shown in Fig. 4.9 (d), phenol concentrations increased from 2 to 13 mg/l at p-NP loading rates varying between 0.96 and 9.13 g/m^3 day. This study showed that p-AP further transformed/degraded to phenol under methanogenic conditions.

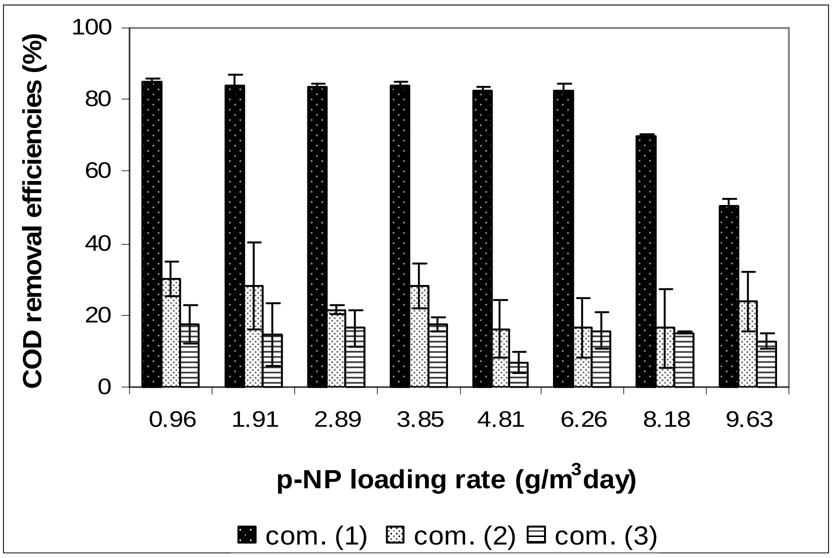


Figure 4.9 The COD variations in compartments of AMBR (a).

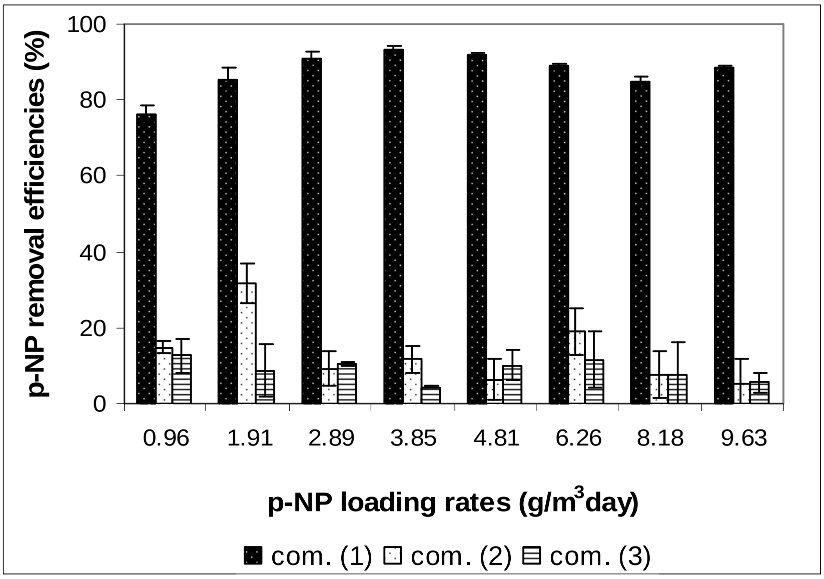


Figure 4.9 The p-NP variations in compartments of AMBR (b).

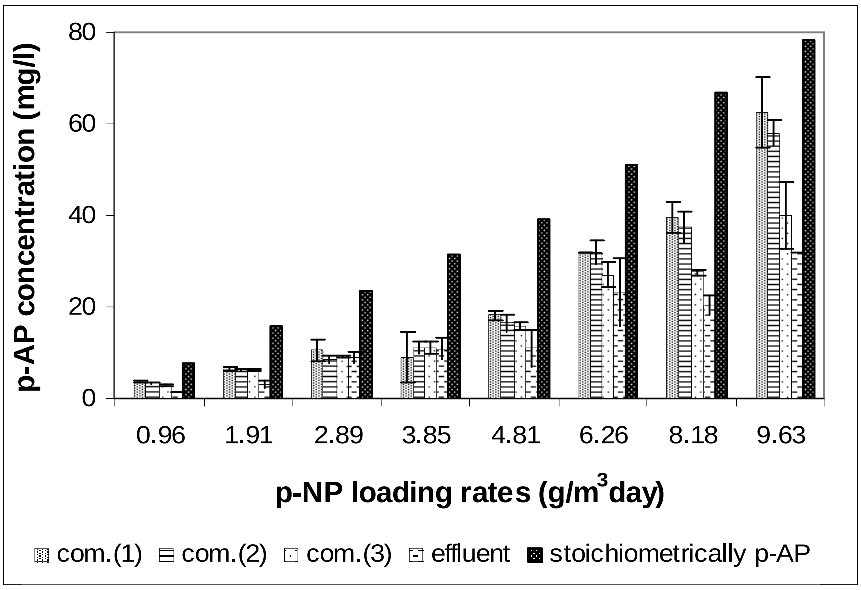


Figure 4.9 The p-AP variations in compartments of AMBR (c).

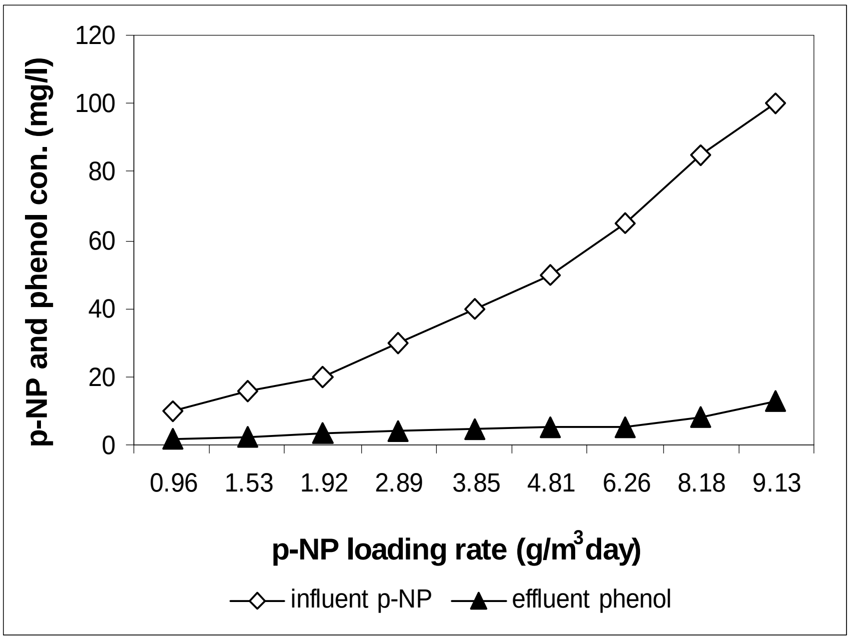


Figure 4.9 The phenol variations in effluent of AMBR (d).

4.2.1.2.6 Variation of pH, Total Volatile Fatty Acid (TVFA), Bicarbonate Alkalinity (Bic.Alk.) and TVFA/Bic.Alk. Ratio in Compartments of the AMBR Reactor at Increasing p-NP Loading Rates. Figure 4.10 indicates the pH, TVFA, bicarbonate alkalinity variations and TVFA/Bic.Alk ratios in the compartments of the AMBR reactor at increasing p-NP concentrations. As shown in Figure 4.10 (a), the influent and effluent pH values varied between 7.6-8.2 and 7.7-8.3 at increasing p-NP concentrations. pH values did not significantly changed in the compartments. The pH values varied between 7.1 and 8.2 in compartment of AMBR at all p-NP concentrations. TVFA concentrations were high in the initial compartment compared the other compartments and effluent samples (See Fig. 4.10 (b)). As shown in this Figure, the TVFA concentrations in compartments and in the effluent of AMBR reactor increased with increasing p-NP concentration (R^2=0.9, df=7, F=26.5, p=0.002). TVFA concentration increased from 55 mg/l to 480 mg/l with increasing of p-NP concentration from 0.96 to 9.63 g/m^{3}day. It was thought that the p-NP or its degradation products, which were accumulated in the reactor contributed to TVFA measurement due to adsorption and slow degradation of inert media such as acetic acid and butyric acid. However TVFA concentrations were below 480 mg/l even is studied as high as p-NP loading rate of 9.63 g/m^{3}day, indicating the stability of AMBR reactor. Stable reactor performance is indicated by the TVFA concentration below 500 mg/l in the effluent of the reactor (Willetts, 1999).

Since CO_2 often exceed other weak acids in aqueous anaerobic systems with microbial activity, sufficient bicarbonate alkalinity must be present to neutralize it and is therefore of prime importance. If the acid concentrations (H_2CO_3 and TVFA) exceed the available alkalinity, the reactor will sour, severally inhibiting microbial activity, especially the methanogens (Speece, 1996). The Bic.Alk. concentrations remained between 3000 and 3300 mg/l in the effluent of AMBR at increasing p-NP concentration (See Fig. 4.10 (c)). The Bic.Alk. concentration in the first compartment was lower than the other compartments. This indicates the utilization of alkalinity to buffer the VFA and CO_2 produced from the anaerobic co-metabolism of p-NP, particularly at high concentrations. TVFA/Bic.Alk. ratio gives necessary information to determine the stability of the anaerobic reactor. If the TVFA/Bic.Alk. ratio is lower than 0.4, the reactor is stable.

When the TVFA/Bic.Alk. ratio is lower than 0.8, the reactor system is moderately stable or unstable (Behling et al, 1997). As shown in Fig. 4.10 (d), this ratio varied between 0.02 and 0.2 in every compartment of AMBR at increasing p-NP concentration. These results indicated that AMBR reactor was stable at increasing p-NP concentration because the TVFA/Bic.Alk. ratios in the effluent and in the compartments were lower than 0.4.

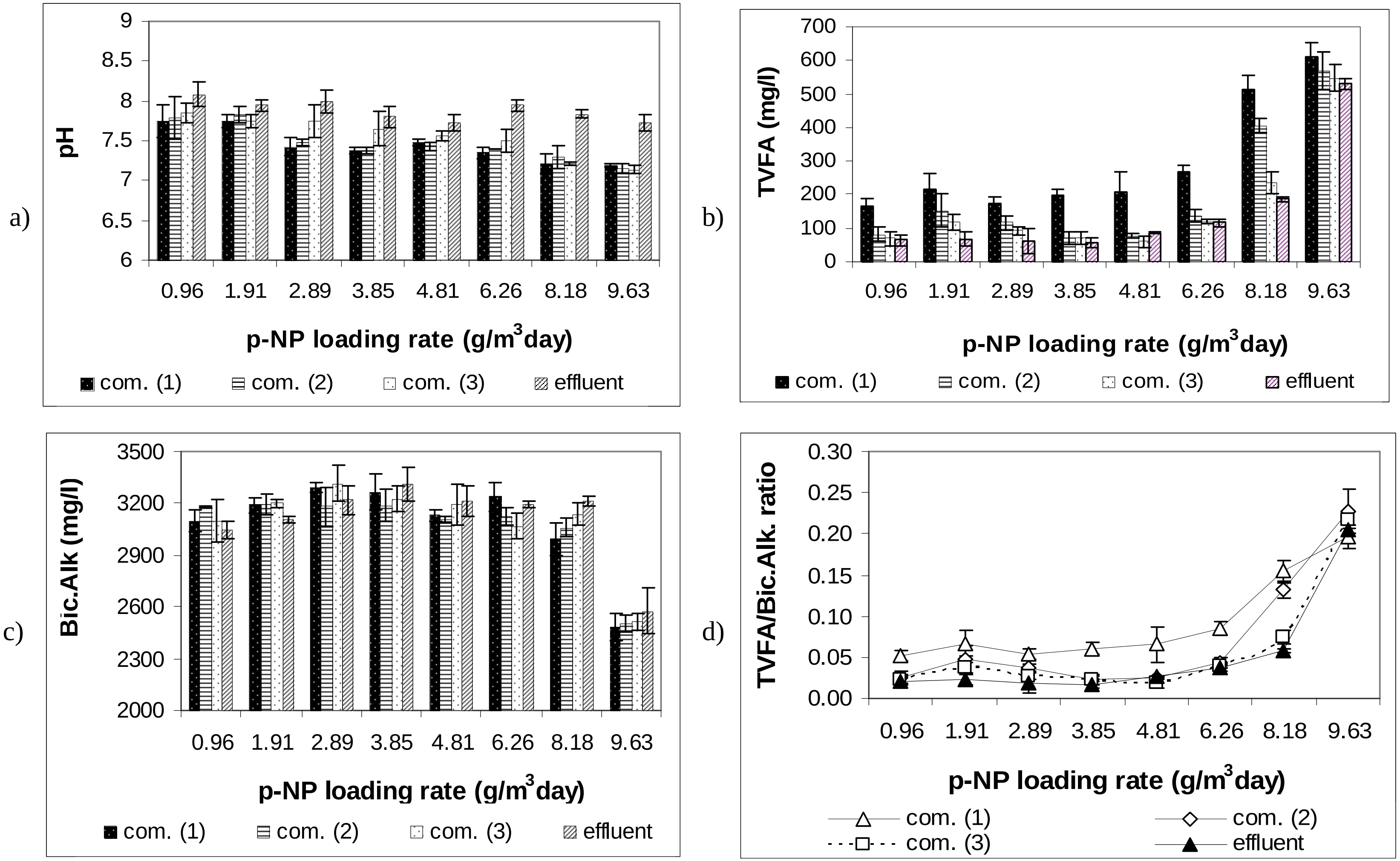


Figure 4.10 The variations of pH (a), TVFA (b), Bic.Alk. (c) and TVFA/Bic.Alk. ratio (d) in AMBR at increasing p-NP loading rates.

4.2.1.2.7 Performance of Aerobic CSTR Reactor and Treatment Efficiencies of Sequential Anaerobic AMBR/Aerobic CSTR Reactor System. Sequential anaerobic/aerobic reactor system consisted of CSTR reactor following the anerobic AMBR reactor. The aerobic reactor was used to removing the residual COD and p-NP entering from the AMBR reactor and mineralization of the intermediate products Figure 4.11 shows the overall COD and p-NP removal efficiencies in sequential AMBR/CSTR reactor system. The COD removal efficiencies were up to 90% until a p-NP loading rate of 8.18 g/m^3 day. The p-NP removal efficiencies were found between 97% and 99% at all p-NP loading rates in sequential AMBR/CSTR reactor system. p-AP produced in anaerobic reactor was mineralized in aerobic reactor. Approximately 91-100% mineralization of p-AP was observed in aerobic phase (See Fig. 4.12). p-AP removal efficiencies were 100% until a p-NP loading rate of 6.26 g/m^{3}day in the aerobic CSTR reactor at a HRT of 6.9 days. After this p-NP loading rate, p-AP removal efficiency decreased to 91 % at p-NP loading rates of 9.63 g/m^{3}day. Our study was compared with the study reported by Melgoza & Buitron (2001). Melgoza & Buitron (2001) found that 98% p-NP removal efficiency was obtained through reaction time of 11.5 h (8 h for the anaerobic phase and 3.5 h for the aerobic one). In this study, the p-NP was transformed to p-AP in the anaerobic phase. A mineralization of 100% of p-AP was observed in the aerobic stage.

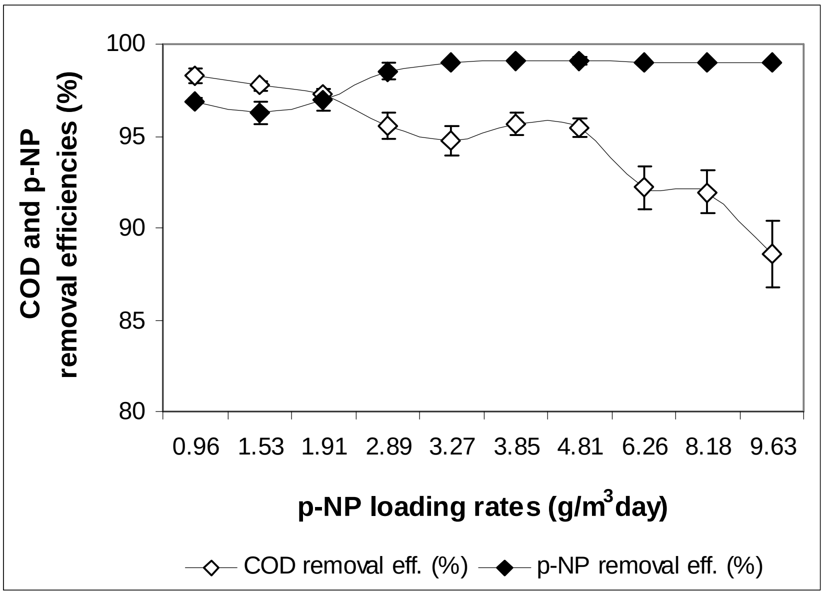


Figure 4.11 The overall COD and p-NP removal efficiencies in sequential AMBR/CSTR reactor system (HRT=16.64 days in AMBR/CSTR reactor system).

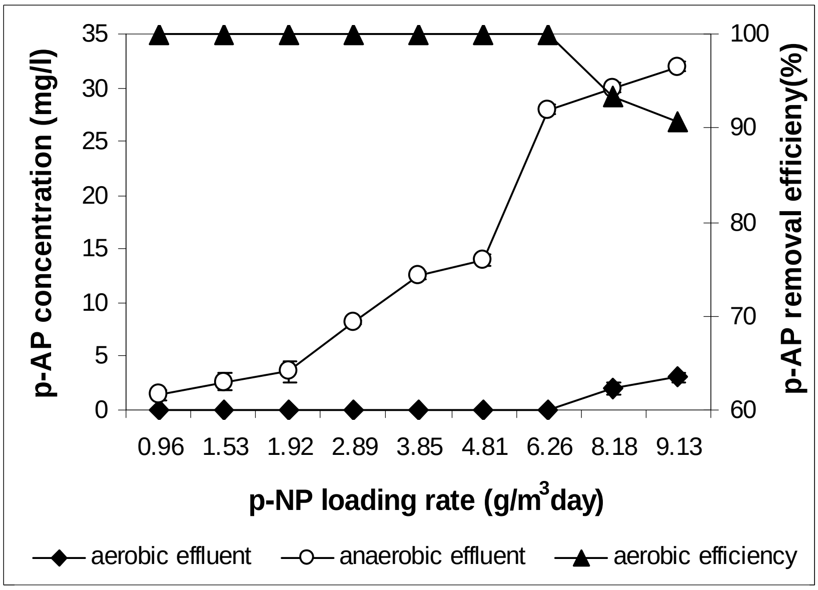


Figure 4.12 The p-AP removal efficiencies in aerobic CSTR reactor (HRT=6.9 days in CSTR).

4.2.1.2.8 Assessment of Toxicity in Sequential Anaerobic AMBR/Aerobic CSTR Reactor System. Toxicity of the effluent of AMBR and aerobic (CSTR) reactor was determined by bioluminescence test using bacteria *Photobacterium phosphoreum* (LCK 480). Table 4.3 shows the inhibition percentages for samples taken from the influent synthetic wastewater containing p-NP of 100 mg/l, effluent of AMBR and aerobic CSTR effluent through continuous operation at a HRT of 10.38 day. As shown in Table 4.3, the inhibition percentage (H) of influent was found as 99% at inhibition time of 30 min. This shows that influent wastewater was toxic according to toxicity evaluation criteria for luminescent bacteria (Lange, 1994). After anaerobic treatment, the inhibition percentage (H) of the effluent of AMBR decreased to 62 % at incubation time of 30 min. This shows that p-NP transformed to less toxic intermediate products under anaerobic conditions. However, the effluent of AMBR was toxic ($20<H<90$) (Lange, 1994). The toxic effect decreased after aerobic treatment. The inhibition percentage (H) of CSTR reactor effluent was found as 19 % at an incubation time of 30 min. This showed that the effluent of CSTR reactor was moderate toxic ($5<H<20$). Therefore, toxicity removal efficiency was found as 80% in sequential AMBR/CSTR reactor system. 1/2, 1/4 1/8, 1/16 and 1/32 dilutions were applied to the samples. According to the dilutions, dilution factors (G_L) were 2, 4, 8, 16 and 32, respectively. For example, if a wastewater is diluted 1/4, G_L is equal to 4. In order to determine the concentration causing 50% inhibition on *Photobacterium phosphoreum*, % inhibition (H) versus G_L values are ploted. Then, the G_L value indicating the IC_{50} value (dilution factor causing 50% inhibition) was found from the axis.

Table 4.3 Toxicity values in sequential AMBR/CSTR reactor system (p-NP= 100mg/l, HRT=10.38 days).

Inhibition H (%)	**Time (min.)**	**Anaerobic Influent**	**Anaerobic effluent**	**Aerobic effluent**
H_5	5	98.1	58.5	18.4
H_{15}	15	98.94	62.2	19.1
H_{30}	30	99.1	62.8	19.8
IC_{50}	G_L	24	2	1

4.2.1.3 Effect of Hydraulic Retention Time (HRT) on Performance of AMBR Reactor

4.2.1.3.1 Effects of HRTs on COD and p-NP Removal Efficiencies in Anaerobic AMBR Reactor. The effect of HRT on the COD and p-NP removal efficiencies are shown in Figure 4.13. HRT decreased from 10.38, 5.19, 3.4, 2.4, 1.5 to 1 day, when the OLR increased step by step from 0.31 to 0.6, 0.93, 1.31, 1.19 and 3.25 kg/m^3 day. 92% COD removal efficiency and 95% p-NP removal efficiencies were obtained at a HRT of 10.38 days and at an influent p-NP concentration of 40 mg/l. The COD and p-NP removal efficiencies decreased from 92% to 82% and from 95% to 86%, respectively with decreased from 10.38 days to 1 day of HRT. The maximum COD and p-NP (E = 90–92% and E=92–95%) removal efficiencies were observed at HRTs varying between 10.38 and 3.4 days. The COD and p-NP removal efficiencies were 82% and 86%, respectively, at a HRT of 1 day. These removal efficiencies were higher that those obtained by Karim & Gupta, (2001). 88% of COD and 59% of p-NP were removed in upflow anaerobic sludge blanked reactor (UASB) at HRT of 30 h and influent NP concentration of 30 mg/l.

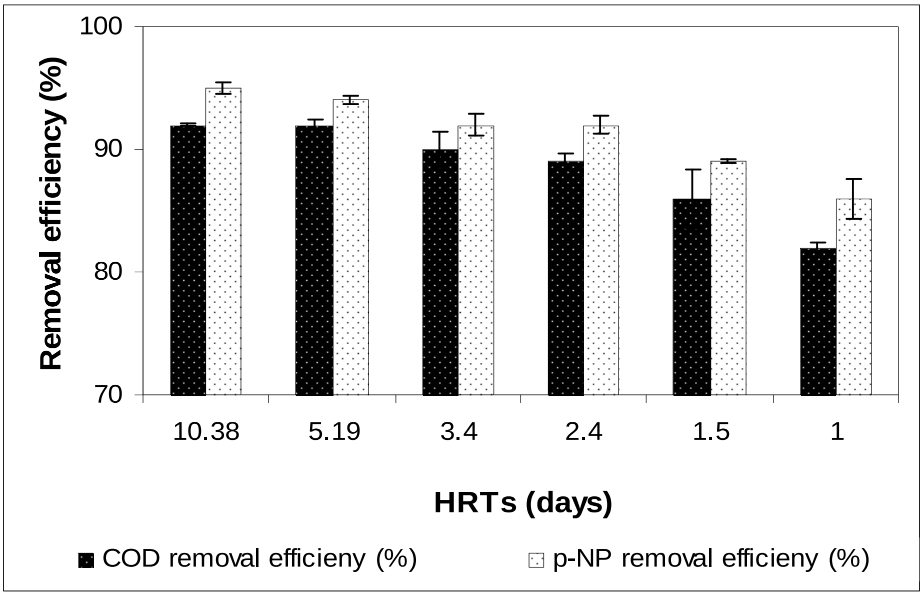


Figure 4.13 The effect of HRTs on the COD and p-NP removal efficiencies in AMBR.

4.2.1.3.2 Effect of HRTs on the Total and the Methane Gas Productions in AMBR Reactor. The variations of the total, methane gas productions and methane gas percentage in AMBR are shown for all HRTs in Figure 4.14. From this figure, it can be seen that daily total and methane gas productions increased as the HRT was decreased. Conversely, methane gas percentages decreased with decreasing HRT. The daily total and methane gas productions increased from 2.16 to 12.25 l/day and from 1.015 to 3.8 l/day, respectively, as HRT decresed from 10.38 days to 1 day. Total gas production was found as 12.25 days at HRT of 1 day (24 h). Total gas production was higher that those obtained by Karim & Gupta (2001). 6.6 l/day was found in upflow anaerobic sludge blanked reactor (UASB) at HRT of 24 h. This can be explained by the high concentrations of biomass in the AMBR reactor due to compartmentalised structure of AMBR (Angenent & Sung, 2001). As shown in the Figure 4.14, methane gas percentage decreased from 47% to 31% when the HRTs were decreased from 10.38 to 1 day. This can be explained by the partial dominancy of acidogenesis compared to methanogenesis at high OLRs or low HRTs in AMBR. If the rate of acid formation exceeds the rate of breakdown to methane, a process unbalance results with decreases in methane content of biogas. On the other hand, the volatile fatty acids converted to the intermediates and end products such as N_2, H_2 instead of methane (data not shown).

The optimum HRTs for maximum methane gas productions (43–47%) varied between 10.38 and 3.4 days. A strong linear correlation between COD removed and methane percentage, COD removal and methane gas production was observed, respectively (R = 0.94; d.f. = 5, F = 35.51, p = 0.03 and R = 0.85, d.f. = 5, F = 11.28, p = 0.01). In this study, the methane yield (m^3 CH_4/kgCOD removed) can be a useful parameter to assess the performance of AMBR. As the treatment of wastewater is directly related to the amount of methane produced, the amount of methane generated per kg of COD stabilized is taken to be an indicator of p-NP and COD stabilization degree. Fig.4.15 shows the variations of methane yields versus HRTs. It was observed that the methane yields decreased from 0.26 to 0.11m^3 CH_4/kg COD, when the HRT were increased from 10.38 days to 1 day (R^2=0.95, df=5, F=40, p=0.003). The methane yield results obtained in this study are higher than those obtained by Uyanik, Sallis, & Anderson, (2002).

Uyanik, Sallis, & Anderson, (2002) found that the methane yield was 0.15 m^3 CH_4/kg COD at an OLR of 0.62 kg COD/m^3 day in an anaerobic baffled reactor (ABR) treating ice-cream wastewater.

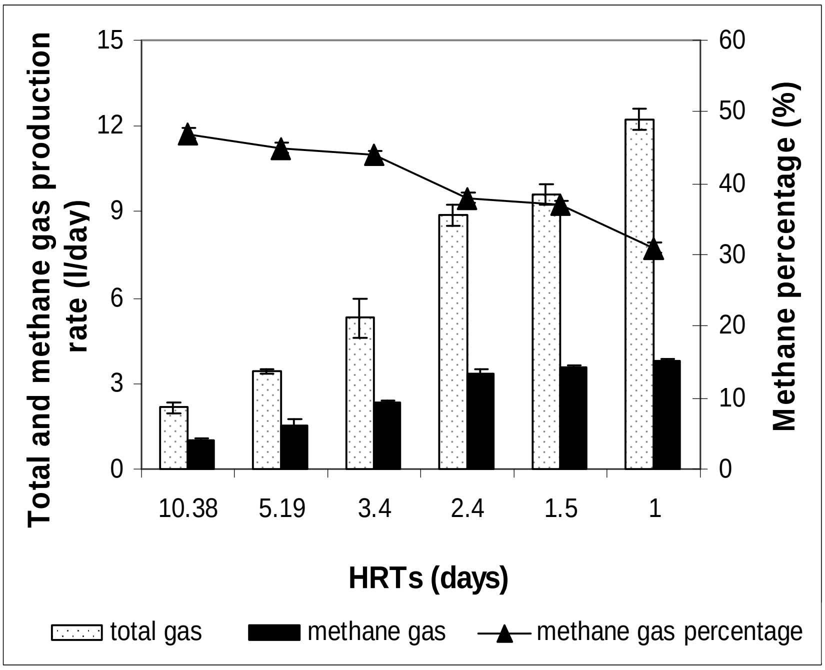


Figure 4.14 The effect of HRTs on total, methane gas production and methane percentage in AMBR.

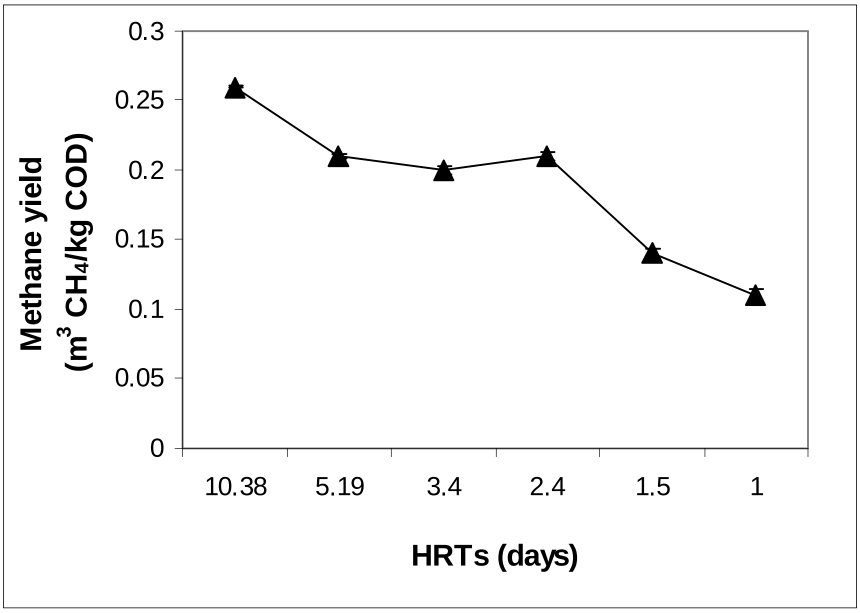


Figure 4.15 Variations of methane yields versus HRTs in AMBR.

4.2.1.3.3 Effect of Compartments on COD and p-NP Removal Efficiencies in AMBR. In order to determine the variations of COD and p-NP in compartments of AMBR reactor, samples were taken from the effluent of each compartment of AMBR reactor and the COD and p-NP concentration variations were measured. Figure 4.16 shows the COD and p-NP concentration variations in compartments of AMBR. As indicated in Figure 4.16 (a), The COD concentrations were quite different in three compartments, indicating that staging had been accomplished. It can be shown that the most of the influent COD was removed in compartment 1 (72–82% of COD). The COD concentration decreased from 940 to 423 mg/l in compartment 1, when the HRT decreased from 10.38 to 1 day. The smaller COD removals (between 13 % and 46%) occurred in compartment 2 and the remaining fraction of influent COD was removed with removal efficiencies varying between 9 % and 22% in compartment 3. Figure 4.16 (b), shows the variations of p-NP in compartments of AMBR. The p-NP concentration increased from 3.6 to 9.3 mg/l in the initial compartment, from 3.2 to 6.5 mg/l in the second compartment, from 2.7 to 5.9 mg/l in the third compartment as the HRT decreased from 10.38 to 1 day, approximately 76-90% p-NP removal efficiencies were achieved in the initial compartment, 14-40 % in second compartment and 10-20% in third compartment when the HRT was decreased from 10.38 to 1 day.

Angenent, Abel, & Sung (2002) investigated the effect of shock OLR at four different HRTs (13, 8,7, 6.5 h) at increasing COD concentrations in AMBR reactor. SCOD removal efficiency was above 60% even during the shock load in the initial compartment**.** Moreover, the performance of the AMBR was above 87% even high shock loading (Angenent, Abel, & Sung, 2002). In our study, SCOD removal efficiency was found as 76% in the first compartment, 13% in the second compartment, and 9% in the third compartment at minimum HRT of 1 day and at a OLR of 86.4 g/m^{3}day**.** Total SCOD removal efficiency was 82% in AMBR at a HRT of 1 day (See Fig.4.13). This shows that compartmentalised anaerobic reactor promote the removal efficiency even OLR as high as 3.25 g/m^{3}day (HRT=1 day).

As seen in Fig. 4.16, the effluent of AMBR reactor exhibit higher treatment efficiencies compared to the final compartment since this final compartment can not serve as an internal clarifier preventing the loss in the effluents. Therefore, the effluent has higher removal efficiency compared to the third compartment. It is important to note that, the samples were taken from the middle part of the final compartment while the effluent samples were withdrawn from the upper part of the compartment with a piping. The length of the piping is 10 cm and the effluent water collected in a bottle with a retention time of 10 min. This probably caused to high removals in the effluent samples compared to the samples taken from the final compartment.

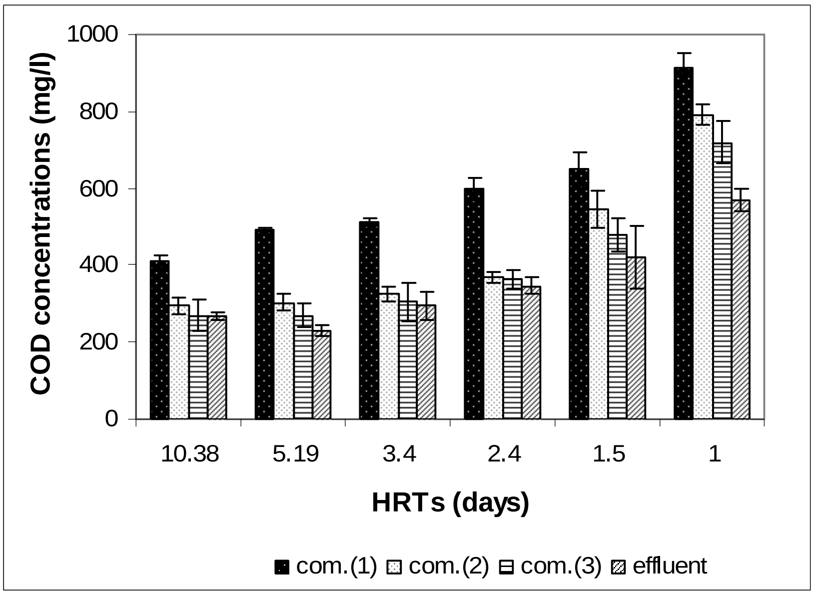


Figure 4.16 Variation of COD in compartments of AMBR (a).

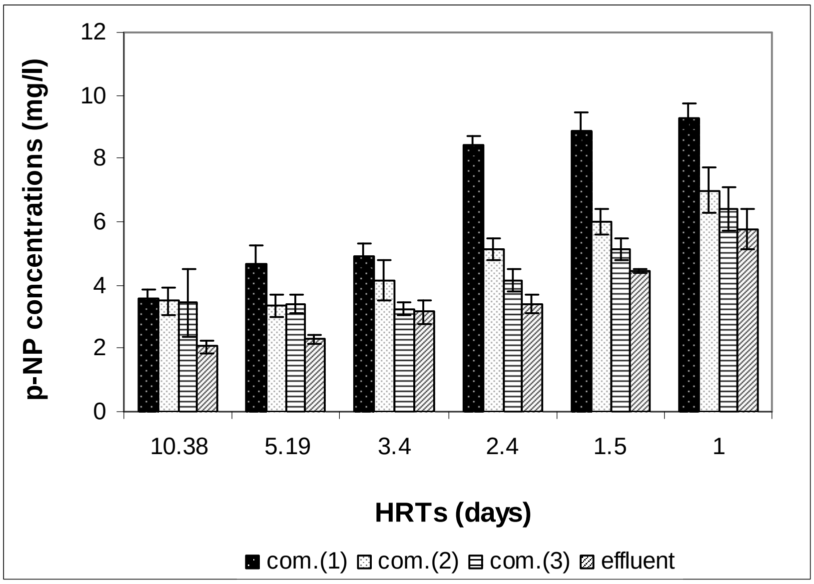


Figure 4.16 Variation of p-NP in compartments of AMBR (b).

4.2.1.3.4 Variations of p-AP and Phenol Concentrations in the Compartments of AMBR. Figure 4.17 shows the variation of p-AP and phenol in compartments of AMBR. The p-AP production was found to be higher in the initial compartment than the other two compartments (See Fig. 4.17 (a)). p-AP concentrations increased from 15 mg/l to 27 mg/l in initial compartment, from 10 to 19 mg/l in the second compartment and from 9.5 to 21 mg/l in the third compartment as the HRT decreased from 10.38 days to 1 day. Stoichiometrically 40 mg/l of p-NP produces 31.4 mg/l of p-AP. On molar basis about 76–90% of p-NP was converted to p-AP in initial compartment and 4–10% of p-NP in subsequent compartments at HRTs varied between 10.38 days and 1 day in whole AMBR. In the present study it was observed that transformation of p-AP to phenol decreased from 50% to 14% as the HRT decreased from 10.38 days to 1 day. Therefore p-AP concentration increased as HRT decreased from 10.38 days to 1 day (See Fig 4.17 (a)). Conversely, phenol concentration decreased as HRT decreased from 10.38 days to 1 day (See Fig.4.17 (b)). Figure 4.17 (b) shows the variation of phenol in compartments of AMBR. As shown in Fig. 4.17 (b), phenol concentration was decreased from 11 mg/l to 4 mg/l in first compartments of AMBR as HRTs decreased from 10.38 days to 1 day. The phenol concentration was higher in the first compartment than the other compartments at all HRTs. Phenol concentration decreased in second and third compartment. This showed the mineralization of phenol in anaerobic reactor.

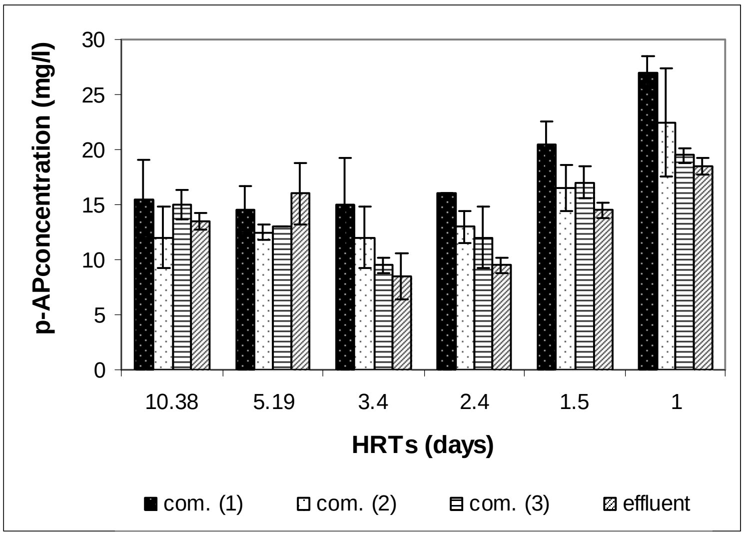


Figure 4.17 The variations of p-AP in compartments of AMBR (a).

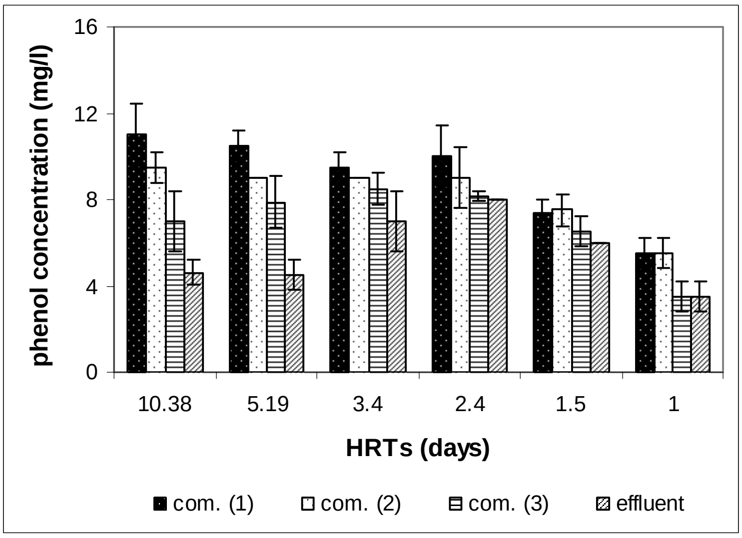


Figure 4.17 The variations of phenol in compartments of AMBR (b).

4.2.1.3.5 Effects of HRTs on pH, TVFA, Bicarbonate Alkalinity (Bic.Alk.) and TVFA/Bic.Alk. Ratio Variations in Compartments of the AMBR. Figure 4.18 shows the variations of pH, TVFA, Bic.Alk. and TVFA/Bic.Alk. ratio in compartments of AMBR reactor at decreasing HRTs. As shown in Fig. 4.18 (a), the influent pH values remained stable at between 7.4 and 7.9 through continuous operation. The pH values varied between 7.5 and 8 in the effluent of AMBR at HRTs varied between 10.38 days and 1 day. These values are between optimum pH values of 6.5 and 8.3 reported by Speece, (1996). From Figure 4.18 (a) shows that the pH values in the first compartment were lower than the other two compartments. The possible reason of the decreases of pH in the first compartment can be explained by the increasing of TVFA levels (See Fig. 4.18 (b)). TVFA concentrations in first, compartment increased from 167 mg/l to 368 mg/l as HRT decreased from 10.38 days to 1 day. However pH values were between optimum values, approximately, 7.2 in the first compartment at all HRTs, because of sodium bicarbonate concentration in the feed water (approximately 5000 mg/l $NaHCO_3$). pH values varied between 7.2 and 7.6 in the second and third compartments at all HRTs. pH values in the effluent were around 7.3 at all HRTs. TVFA concentrations decreased in second and third compartment and effluent at all HRTs. This caused rising of pH values in the second and third compartments. TVFA concentrations decreased from 188 mg/l in the first compartment to 143 mg/l in the second compartment and to 39 mg/l in the third compartment at an HRT of 1 day. When HRT was decreased from 10.38 days to 1 day, the TVFA concentrations in first, second and third compartments were 353, 310 and 290 mg/l, respectively. The TVFA concentration in the effluent increased from 23 to 177 mg/l as HRT decreased from 10.38 to 1 day. Our studies exhibit similar results with the studies performed by Angenent, Abel, & Sung (2002) in AMBR reactor at a HRT of 3h. In this study, VFA concentration was high in the first compartment than other compartments. VFA concentrations were found as 1859 mg/l in the first compartment, 1388 mg/l in the second compartment, 774 mg/l in the third compartment, 432 mg/l in the fouth compartment and 353 mg/l in final compartment at shock OLR of 50 kgCOD/m^3day at HRT of 3 h. However pH values were between 6.2 and 6.9. Karim & Gupta, (2001) found that TVFA and pH values in the effluent were 971 mg/l and 8.4, respectively in the effluent of UASB reactor treated 30 mg/l of p-NP of at a OLR of 4 kg COD/m^{3}day and at a HRT of 12 h. This indicated the stabilty of AMBR reactor compared to UASB reactor.

The bicarbonate alkalinity (Bic.Alk.) in the feed was required to buffer the media to provide the favorable conditions for conversion of substrate to methane (Speece, 1996). Bic.Alk. in the feed wastewater was obtained 5000 mg/l of $NaHCO_3$, which is 1.6 times higher than COD in the feed.With carbohydrate wastes the alkalinity requirement is 1.2–1.6 g alkalinity as $CaCO_3$/g influent COD which is sufficient to maintain the pH above 6.6 reported by Speece, (1996). Figure 4.18 (c) shows Bic.Alk. concentrations in the compartment and effluent of AMBR at different HRTs. Bic.Alk. concentrations in the feed wastewater was around 3000-3200 mg $CaCO_3$/l at all HRT. Bic.Alk. concentration in the first compartment was lower than the other compartments due to higher TVFA. Bic.Alk. concentration in the first compartment decreased from 3180 mg $CaCO_3$/l to 2800 mg $CaCO_3$/l with increasing of TVFA concentration from 167 mg/l to 360 mg/l as the HRT decreased from 10.38 days to 1 day. Bic.Alk. concentrations in the second and third compartment varied between 3200-3300 mg $CaCO_3$/l until a HRT of 2.4 days. Bic.Alk. concentration was around 2900 mg $CaCO_3$/l in compartments 2 and 3 at lower HRTs such as 1 day. This caused high TVFA concentration in compartments of AMBR at lower HRTs. The effluent Bic.Alk. concentrations were between 3000-3300 mg $CaCO_3$/l at all HRTs.

Fig. 4.18 (d) showed the TVFA/ Bic.Alk. ratios in the compartments and in the effluent of AMBR. This ratio changed between 0.04 and 0.13 in compartment 1, between 0.03 and 0.11 in compartment 2 and between 0.01 and 0.10 in compartment 3 and between 0.02 and 0.09 in the effluent of AMBR at all HRTs. TVFA/ Bic.Alk. ratios were lower than 0.4 in the compartments and in the effluent of AMBR at all HRTs. This shows the stability of the AMBR reactor as reported by Behling et al., (1997).

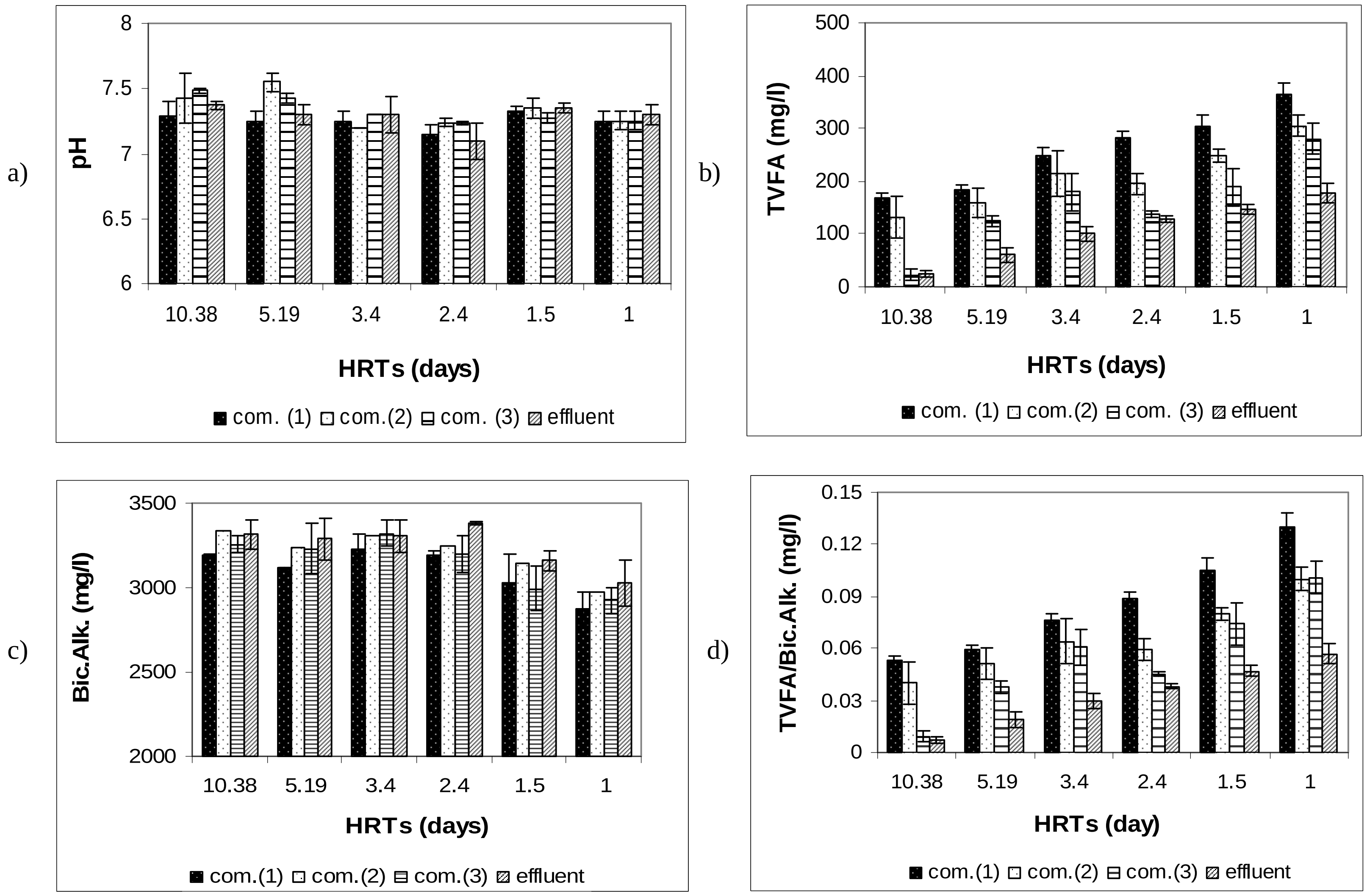


Figure 4.18 The variations of pH (a), TVFA (b), Bic.Alk. (c) and TVFA/Bic.Alk. rations (d) in compartments of AMBR at decreasing HRTs.

4.2.1.3.6 Specific Methanogenic Activity (SMA) and F/M Ratio Variations in AMBR at Different HRTs. Figure 4.19 shows the SMA values of sludge taken from middle compartment of AMBR during continuous operation of AMBR at different HRTs. The SMA is an indicator of methanogenic activity of biomass without substrate limiting factor. As shown in Figure 4.19., SMA values decreased from 1.08 g COD-CH_4/g VSS day to 0.6 g COD-CH_4/g VSS day when HRT decreased from the 10.38 days to 1 day (approximately 40% reduce was observed in SMA). SMA was around 0.85 g COD-CH_4/g VSS day at high HRTs such as 5.19 days and 3.4 days. SMA increased 0.96 g COD-CH_4/g VSS day at a HRT of 2.4 days. This loading was tolerated by the granules, resulting in recoveries in the SMA. After this HRT, SMA values decreased to 0.79 g COD-CH_4/g VSS day at a HRT of 1.5 day and 0.64 g COD-CH_4/g VSS day at a HRT of 1 day. The reason of this could be explained with high flow rates and OLRs which decrease the activity of methanogens. Afterwards, it can be concluded that methanogenic activity decreased with decreased HRTs. Donlon et al., (1996) reported a SMA od 0.87 g COD-CH_4/g VSS day in an UASB treating 260 mg/l p-NP at a HRT of 0.33 days.

Figure 4.20 shows the F/M ratio variations in AMBR at different HRTs. F/M ratio increased from 0.006 to 0.02 (day^{-1}) as HRT decreased from 10.38 days to 1 day (R^2=0.92, f=24.36, p=0.008, df=5). VSS concentration increased from 24 g/l to 44 g/l as HRT decreased from 10.38 days to 1 day. F/M ratio variations depends on COD loading rate (R^2=0.99, f=605, p=0.002, df=5) and VSS concentration in reactor (R^2=0.96, f=47.9, p=0.018, df=5).

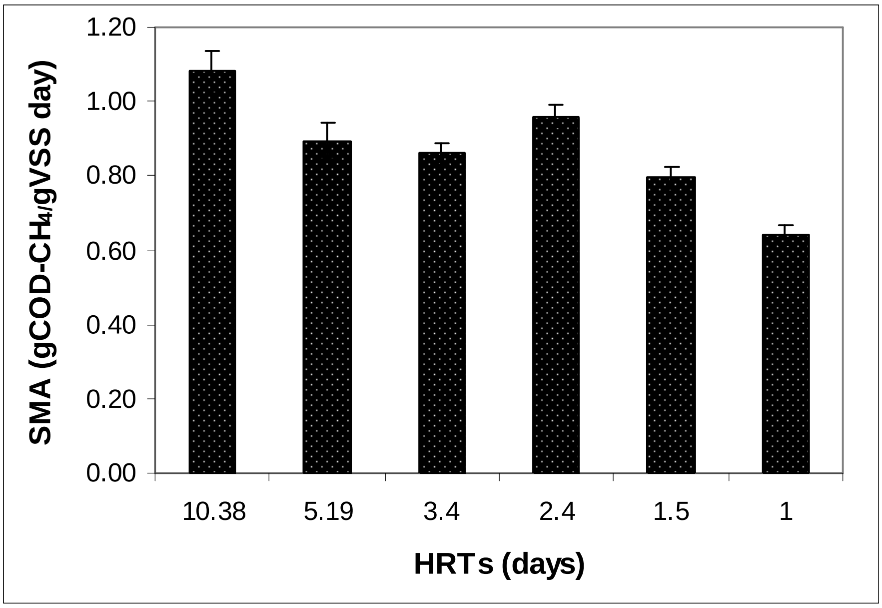


Figure 4.19 SMA values in AMBR at different HRTs.

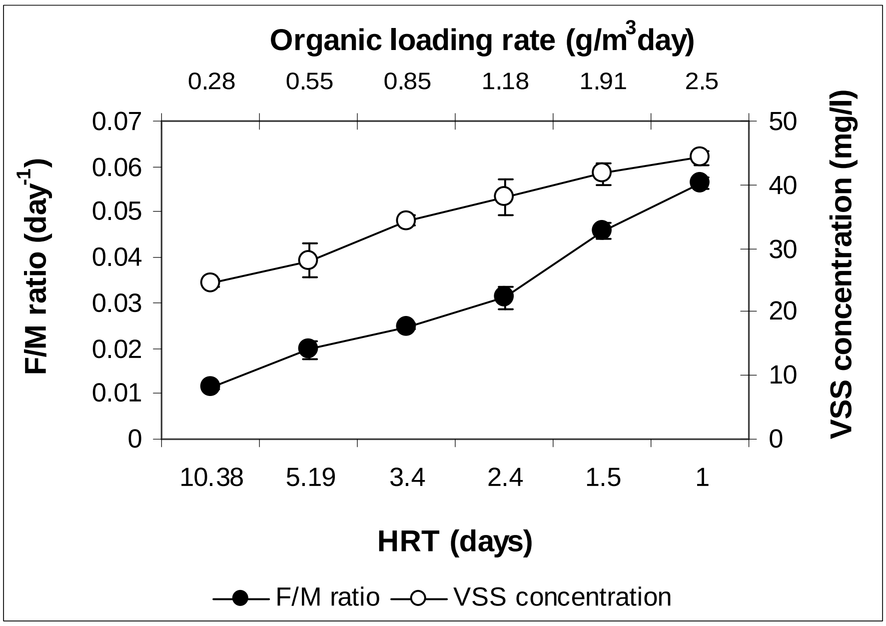


Figure 4.20 F/M ratios and VSS concentrations variations in AMBR at different HRTs.

4.2.1.3.7 Performance of Sequential Anaerobic AMBR/Aerobic CSTR Reactor System. Figure 4.21 shows the overall COD and p-NP removal efficiencies in sequential AMBR/CSTR reactor system. The maximum COD and the p-NP removal efficiency in sequential AMBR/CSTR reactor system were measured as 98% and 96% at a HRT of 17.3 days, respectively. COD and p-NP removal efficiencies were 94% and 92 % at a minimum HRT of 1.7 days in overall reactor system, respectively. Total COD and p-NP removal efficiencies decreased from 99% to 94% and from 96% and 92% as the HRT decreased from 17.3 days to 1.7 days in sequential anaerobic AMBR/aerobic CSTR reactor system. COD and p-NP removal efficienies were above 93% and 92%, respectively, at all HRTs in sequential AMBR/CSTR reactor system

The biotransformation of p-NP to p-AP was observed in the reductive anaerobic phase. A 79%–92% of the p-NP transformed to p-AP in anaerobic phase (See Table 4.4). The p-AP was mineralized to phenol by anaerobic bacteria in AMBR. A mineralization of 100% of p-AP was found in the aerobic phase (See Table 4.4). In this study, the generation of ammonia (NH_4- N) was observed during the anaerobic degradation of p-AP in AMBR. The p-AP, phenol and NH_4-N produced in anaerobic phase converted to nitrite (NO_2), nitrate (NO_3) and CO_2 as end products under aerobic conditions. Table 4.4 shows a nitrogen balance through p-NP degradation. As is known, a nitrogen source is essential for microbial growth for aerobic biological systems. In this study, NH_4-N produced through anaerobic phase was used as the nitrogen source for oxidation of NO_2-N to NO_3-N by aerobic microorganisms. This consumption suggests that there was nitrification process converting the NH_4-N to NO_2-N and NO_3-N in CSTR reactor. As shown in Table 4.4, a high amount of NH_4-N produced by the anaerobic bacteria was removed by the aerobic bacteria with removal efficiencies of 42% and 81%. A 40 mg/l of p-NP converted to 14 mg/l of p-AP, 4.6 mg/l of phenol and 8.9 mg/l of ammonia under anaerobic conditions. The ammonia concentration decreased to 1.9 mg/l, then converted to 11 mg/l of NO_2-N and 10 mg/l of NO_3-N at an OLR of 0.31 kg/m^{3}day under aerobic conditions for Run 1 (See Table 4.4). The mass balance between 40 mg/l of p-NP and inter metabolites produced in the anaerobic AMBR reactor effluent is as follows: 14 mg/l of p-AP + 4.6 mg/l of phenol + 8.9 mg/l of NH_4-N + 2.27 mg/l remaining p-NP is equal to 29.7 mg/l. The difference between initial p-NP and the sum of produced products showed that the presence of other inter metabolites products. A 40 mg/l of p-NP converted to 13 mg/l of p-AP, 4.7 mg/l of phenol and 9.8 mg/l of ammonia under anaerobic conditions in Run 6. Then the ammonia concentration decreased to 5.6 mg/l under aerobic conditions via nitrification. In the next step ammonia converted to 12 mg/l of NO_2-N and 5 mg/l of NO_3-N at OLR of 3.25 kg/m^3day under anaerobic conditions (See Table 4.4). The mass balance between 40 mg/l of p-NP and inter metabolites are as follows under anaerobic conditions:

15 mg/l of p-AP + 13.5 mg/l of phenol + 5.17 mg/l of NH_4-N + 5.6 mg/l of remaining p-NP are equal to 40.2 mg/l. The mass balance between 40 mg/l of p-NP and inter metabolites produced in the anaerobic AMBR reactor was tabulated in Table 4.4. The results showed that 22 mg/l of p-AP + 7 mg/l of phenol + 6 mg/l of NH_4-N are equal to 35 mg/l p-NP in the first compartment with the exception of remaining p-NP in Run 1 under anaerobic conditions. It is important to note that NH_4-N was completely nitrified to NO_3-N. The reason for this could be the complete nitrification due to the presence of high number of nitrifying bacteria in the aerobic biomass. The 8.9 mg/l of NH_4-N was converted to 9 mg/l of NO_2-N and 10 mg/l of NO_3-N in aerobic reactor at an OLR rate of 0.31 kg/m^3 day. Similarly the 9.8 mg/l of NH_4-N was converted to 9 mg/l of NO_2-N and 5 mg/l NO_3-N in aerobic reactor at an OLR rate of 3.25 kg/m^3 day. According to Turkish Water Pollution Control (1988) the discharge limits for TN, NH_4-N and NO_2-N are 40, 0.02–5 and 10 mg/l, respectively, for industries which produce nitroorganic compounds (Turkish Water Pollution Control Regulation, 1998). As shown there is not any limitation for NO_3-N. In this study the NO_2-N was lower than the Turkish discharge standards.

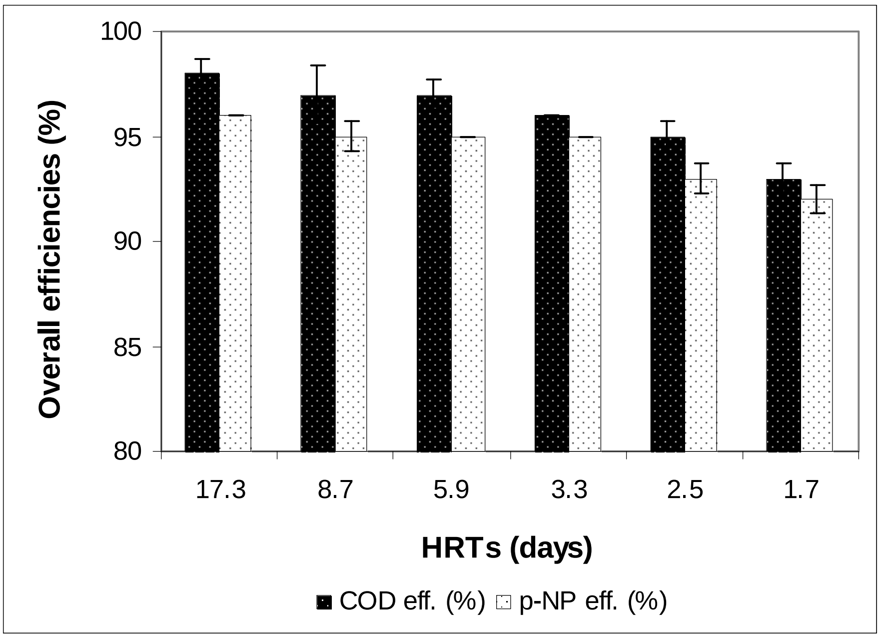


Figure 4.21 The total performance of sequential AMBR/CSTR reactor system.

Table 4.4 Nitrogen balance for the p-NP degradation in sequential AMBR/CSTR reactor system.

Period (days)		**HRT**	**OLR**	**p-NP (mg/l)**		**p-AP (mg/l)**		**Phenol (mg/l)**		**NH_4-N (mg/l)**		**NO_2-N (mg/l)**		**NO_3-N (mg/l)**	
				Inf.	**Eff.**	**Inf.**	**Eff.**	**Inf.**	**Eff.**	**Inf.**	**Eff.**	**Inf.**	**Eff.**	**Inf.**	**Eff.**
Anaerobic (AMBR) reactor															
Run 1	35	10.38	0.31	40	2.3	0	14	0	4.6	0	8.9	0	0	0	0
Run 2	33	5.19	0.60	40	2.3	0	14	0	6.8	0	11	0	0	0	0
Run 3	27	3.4	0.93	40	3.2	0	13	0	4.3	0	10	0	0	0	0
Run 4	34	2.4	1.31	40	3.4	0	15	0	5.4	0	9.6	0	0	0	0
Run 5	32	1.5	2.14	40	4.5	0	12	0	8.6	0	11	0	0	0	0
Run 6	25	1	3.25	40	5.6	0	13	0	4.7	0	9.8	0	0	0	0
Aerobic (CSTR) reactor															
Run 1	35	6.92	0.03	2. 3	1.8	14	0	4.6	2.9	8.9	1.9	0	9	0	10
Run 2	33	3.46	.07	2.3	1.7	14	0	6.8	4.6	11	3.0	0	8	0	13
Run 3	27	2.25	0.12	3.2	2.0	13	0	4.3	3.2	11	2.4	0	8	0	12
Run 4	34	1.59	0.21	3.4	2.4	15	0	5.4	3.3	9.6	1.8	0	8	0	13
Run 5	32	0.99	0.35	4.5	3.2	12	0	8.6	4.2	11	4.8	0	10	0	9
Run 6	25	0.7	0.79	5.6	3.5	13	0.5	4.7	4.3	9.8	5.6	0	9	0	5
Anaerobic (AMBR)/aerobic (CSTR) reactor system															
Run 1	35	17.3	0.31	40	1.8	0	0	0	2.9	0	1.9	0	9	0	10
Run 2	33	8.65	0.60	40	1.7	0	0	0	4.6	0	3.0	0	8	0	13
Run 3	27	5.65	0.93	40	2.0	0	0	0	3.2	0	2.4	0	8	0	12
Run 4	34	3.99	1.31	40	2.4	0	0	0	3.3	0	1.8	0	8	0	13
Run 5	32	2.49	2.14	40	3.2	0	0	0	4.2	0	4.8	0	10	0	9
Run 6	25	1.7	3.25	40	3.5	0	0.5	0	4.3	0	5.6	0	9	0	5

OLR= Organic loading rate (g/m^3day); HRT= Hydraulic retention time (day); Inf.= Influent; Eff= Effluent

4.2.1.3.8 Assessment of Toxicity of Sequential Anaerobic AMBR/Aerobic CSTR Reactor System. In this study, the toxicity of effluent of AMBR and aerobic (CSTR) reactor were determined by bioluminescence test using bacteria *Photobacterium phosphoreum* (LCK 480) and by *Daphnia magna test*. Table 4.5 (a) shows the inhibition percentage of 5^{th}, 15^{th}, 30^{th} in samples taken from the influent synthetic wastewater containing p-NP of 40 mg/l, from the effluent of anaerobic and aerobic reactor through continuous operation at a HRT of 1 day. LUMIStox test is an acute toxicity test. Therefore the toxicity results of this test could be obtained in the short time (maximum 30 min). As shown in Table 4.5 (a), the inhibition percentage (H) of influent was found as 98% at an incubation time of 30 min. These result showed that wastewater containing 40 mg/l of p-NP was toxic due to 98% inhibition was observed. After anaerobic treatment, the effluent toxicity decreased to 58.1% at incubation time of 30 min as shown in Table 4.5 (a). This shows that p-NP transformed to less toxic intermediate products under anaerobic condition. On the other hand, intermediate products such as p-AP and phenol produced under anaerobic conditions were less toxic of its corresponding p-NP. After aerobic treatment, toxicity decreased from 58% to 19 % in aerobic effluent at a incubation time of 30 min. The aerobic reactor effluent was moderate toxic exhibits possible toxicity However it is imported to note that the toxicity decreased from anaerobic to aerobic reactor. The parent organic chemical is lower toxic than the intermediate products.

Table 4.5 (b) shows the *Daphnia magna* toxicity test results for samples taken from the influent synthetic wastewater containing p-NP of 40 mg/l and the effluent of anaerobic AMBR reactor at a HRT of 5 days. *Daphnia magna* test is accepted as acute toxicity test. Results were expressed as mortality percentage of the *Daphnids.*

After the test samples contining p-NP was diluted, the experiments were carried out using 10 *daphnids. The Daphnids* was added to into every one test vessel at beginning time (t=0). After 24 h of incubation time, IC_{50} value (the concentration inhibited 50% of *daphnia magna*) was found as 4 mg/l in feed wastewater diluted 10 times. This showed that the feed wastewater was toxic for *Daphnids*.

In other words, If the influent wastewater is diluted 10 times, 50% of *Daphnids* was dead. If influent wastewater is diluted 20 times, it was not observed toxicity. The sample taken from the effluent of the anaerobic reactor were diluted 1, 4/5, 3/5, 2/5 and 1/2 ratios. IC_{50} value (50% inhibition of *Daphnids)* was 21.6 mg/l in the effluent sample diluted at a ratio of 4/5. The other dilution ratios do not show any mortality effect of *Daphnid*. The p-NP and p-AP concentrations in the effluent of anaerobic were found as 3.2 mg/l and 24 mg/l, respectively through a HRT of 5 days. This shown that anaerobic reactor decreased the toxicity of the influent and produced less toxic intermediates product than the influent.

Table 4.5 Toxicity values in AMBR/CSTR reactor system (p-NP=40 mg/l, HRT=3.4 days) (a).

Inhibition H (%)	**Time (min.)**	**Anaerobic influent**	**Anaerobic effluent**	**Aerobic effluent**
H_5	5	92.58	53.5	16.43
H_{15}	15	95.94	55.4	19.11
H_{30}	30	98.1	58.2	19.8

Table 4.5 Toxicity values in the influent and effluent of AMBR (p-NP=40 mg/l, HRT=5 day) (b).

	Anaerobic influent		**Anaerobic effluent**		
Dilution ratio	**Daphnia magna number**		**Dilution ratio**	**Daphnia magna number**	
	First start	**24 hours**		**First start**	**24 hours**
1	10	0	**1**	10	0
1 / 2	10	0	**4/5**	10	5
1/ 10	10	5	**3/5**	10	10
1/15	10	7	**1/2**	10	10
1/ 20	10	10	**1/4**	10	10

4.2.1.3.9 General Discussion. The sensitivity of the methanogens to pH coupled with the VFAs which are the intermediates of the anaerobic stabilization of organic matter and resulting in a negative response by anaerobic system (Speece, 1996). As shown in Figure 4.10 (b), TVFA concentration increased from 55 mg/l to 550 mg/l as p-NP concentration increased from 40 to 100 mg/l. However the effluent pH values remained between 7.7 and 8.3, which was between optimal values for anaerobic degradation at increasing p-NP concentrations. When the TVFA concentration in the effluent increased from 25 to 182 mg/l with decreasing of HRT from 10.38 to 1 day, the pH values was between optimal values. The optimal pH could be explained by the neutralization of hydrogen anion released from the volatile fatty acid together with the carbonates dissociated from the carbonic acid and also from the reserved Bic.Alk. in AMBR reactor (Speece, 1996).

The TVFA concentrations in the initial compartment of AMBR was higher that the other compartments. However, pH values were between optimum values at all p-NP loading rate and HRTs (See Fig.4.10 (a)(b) and Fig.4.18 (a)(b)). The TVFA concentrations in the third compartment decreased due to TVFA consumption. Meanwhile the effluent quality improved during the flow pattern of the AMBR reactor as shown by VFA levels in the final compartment. These results could be attributed to the compartmentalisation of the AMBR reactor and reversing of flow direction for sufficient contact between p-NP and biomass.

Similarly the p-NP and COD concentrations in the initial compartments of the AMBR reactor increased while the AMBR reactor was beneficial in promoting a complete removal of the aforementioned parameters in the final compartment of AMBR reactor. The initial compartment of the AMBR reactor achieved the highest removal. The final compartment also acts as a buffer for the removal of VFA and remaining COD as reported by Angenent, Banik & Sung, (2001). In the initial compartment achieved high COD and p-NP removals at increasing p-NP loading rates and degreasing HRTs (Fig.4.9. (a)(b) and 4.16. (a)(b)). The AMBR reactor showed no noticeable deterioration of effluent quality during the continuous operation in which the p-NP loading rate increased from 0.96 to 9.13 g/m^{3}day and the HRT decreased from 10.38 days to 1 day. This study shows that the compartmentalized anaerobic systems have intrinsic characteristics that provide stability even if studied at high p-NP concentrations and lower HRTs in AMBR reactor. In this study, near 56% COD and 90% p-NP removal efficiency was observed at maximum p-NP concentration of 100 mg/l in AMBR reactor. COD and p-NP removal efficiencies were found as 89% and 99%, respectively, in sequential AMBR/CSTR reactor system at a p-NP concentration of 100 mg/l. 82% COD and 86% p-NP removal efficiency was observed at a minimum HRT of 1 day and a p-NP of 40 mg/l in AMBR reactor. Total COD and p-NP removal efficiencies were found as 94% COD and 92%, respectively. The results obtained in this study are higher than the data obtained by Karim & Gupta (2001). Karim & Gupta (2001) found 70% p-NP removal efficiency in an upflow anaerobic sludge blanket reactor at an influent p-NP concentration of 30 mg/l and a HRT of 30 h. In another study performed by Uberoi & Bhattacharya (1997) found that 20 mg/l of p-NP concentration was treated in a batch biofilter under sequential anaerobic/aerobic environmental with 95% efficiency. In this study found that p-NP having high toxicity (98% inhibition after 30 min) transformed to p-AP having lower toxicity (58% inhibition after 30 min) in AMBR reactor. The p-AP mineralized to CO_2 and H_2O in aerobic CSTR reactor. A multi-compartment AMBR was ideal for treating toxic wastewaters and reduced possibly the inhibition of methanogen bacteria.

4.2.2 The Removal of p-NP in Anaerobic Baffled Reactor (ABR) and Sequential ABR/CSTR Reactor System

4.2.2.1 Start-up of Anaerobic Baffled Reactor (ABR)

The ABR reactor was operated through 60 days without p-NP under steady-state conditions to acclimate the granular sludge to ABR reactor. Figure 4.22 shows the COD removal efficiencies in the ABR during the start-up period. The COD removal efficiency was 20% at the operation time of 7 days. The COD removal efficiency was 86% at operation time of 30 days. The COD removal efficiencies remained stable 94% after operation period of 45 days. Figure 4.23 shows the methane gas percentages in the ABR during the start-up period.The methane gas production and methane percentage were approximately 24 mg/l and 10% at the beginning of the start-up period (operation time between 0 and 10 days). The methane gas production and methane percentage reached 1120 ml/day and 55%, respectively at operation time of 7 days. The daily methane gas production and methane percentage remained stable at 1500 ml/day and 60%, respectively, after 45 days of the start-up period.

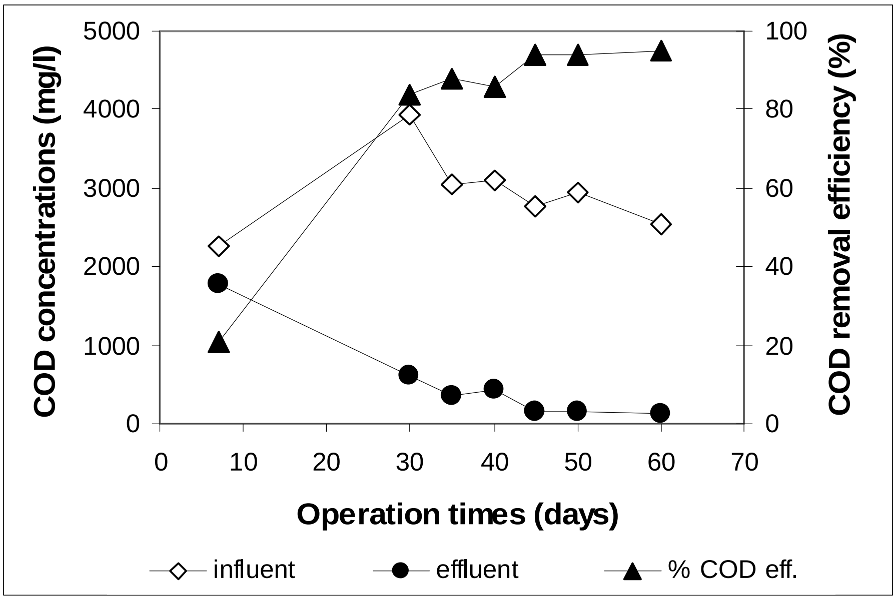


Figure 4.22 COD removal efficiencies in the ABR during the start-up period in ABR.

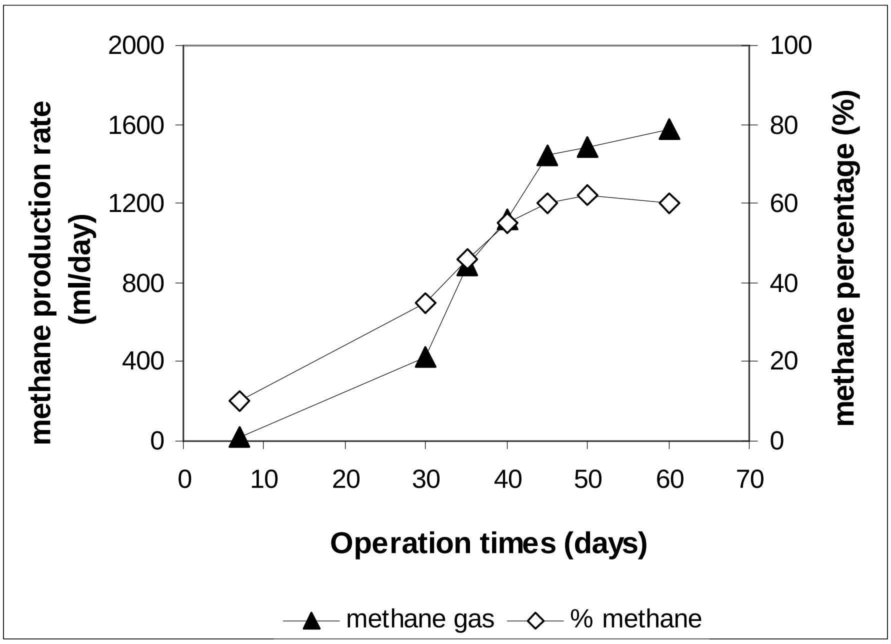


Figure 4.23 Methane gas production and methane percentages in the ABR during the start-up period in ABR.

4.2.2.2 Effect of Increasing p-Nitrophenol Loading rates on Performance of ABR Reactor

4.2.2.2.1. Effect of Increasing p-Nitrophenol Loading Rates on the COD Removal Efficiencies in ABR Reactor. In this study, the effect of increasing p-NP concentrations on COD removal efficiencies was investigated in ABR. The operation of the ABR with p-NP was started at an influent p-NP concentration of 10 mg/l (p-NP loading rate of 0.96 g/m^3 day), and then p-NP concentration was subsequently increased from 20, 35, 45, 85, 100, 140, 250, 350, 500, 600, to 700 mg/l with increasing of p-NP loading rates from 1.94, 3.4, 4.36, 8.32, 9.7, 13.6, 24.2, 33.9, 48.5, 58.2 to 67.9 g/m^{3}day. The effect of p-NP loading rate on the COD removal efficiencies in ABR was shown in Figure 4.24. Although the influent COD concentration was kept constant at 3000 mg/l with glucose, the influent COD concentrations increased with increasing p-NP concentration since p-NP give additional COD to synthetic wastewater. The influent COD concentration was 2965 mg/l at a p-NP concentration of 10 mg/l while it was measured as 4425 mg/l at p-NP concentration of 700 mg/l. The COD removal efficiency was 94% at an initial p-NP loading rate of 0.96 g/m^{3}day introduced to ABR. The effluent COD concentration was measured as 179 mg/l. The COD removal efficiencies were above 90% until a p-NP loading rates of 33.9 g/m^{3}day applied to the reactor. After this loading rate, the COD removal efficiencies started to drop and were measured as 79% at a p-NP loading rate of 67.9 g/m^{3}day resulting in a COD concentration of 926 mg/l in the effluent. The optimum p-NP loading rate and p-NP concentration were found as 8.32 g/m^{3}day and 85 mg/l, respectively, for maximum COD removal efficiency of 94%.

High p-NP loading rates reduced metabolic activity of methanogens due to inhibition of p-NP (IC_{50}=27.7 mg/l). Furthermore, the breakdown products such as p-AP and phenol of p-NP were not ultimately metabolized and caused residual COD. Hutnan et al., (1999) compared to anaerobic UASB, hybrid and baffled reactors. This study showed that anaerobic baffled reactor give higher COD removal efficiency than the other reactors.

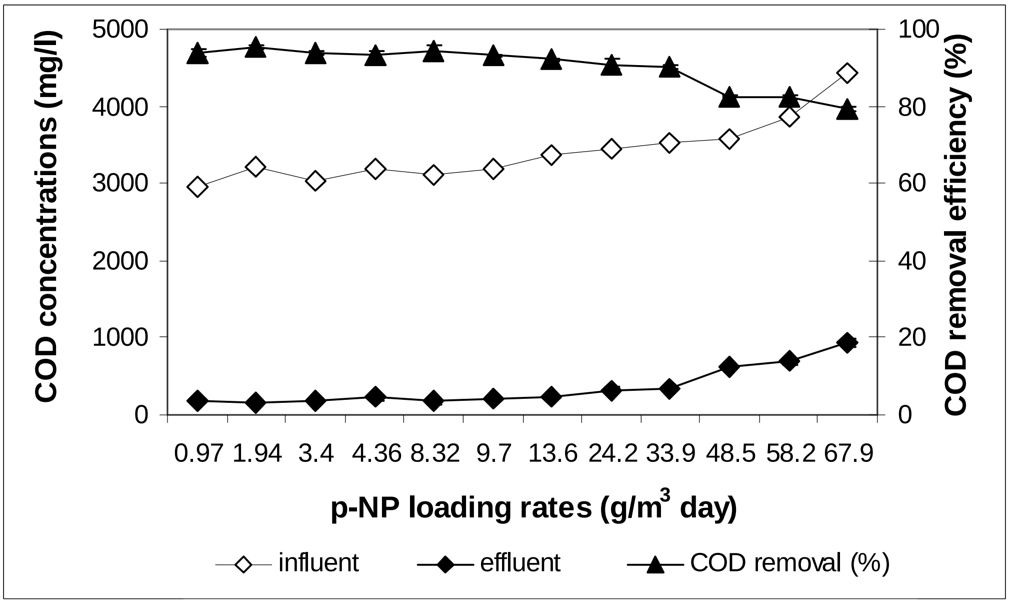


Figure 4.24 Effect of p-NP loading rate on COD removal efficiencies in ABR reactor.

4.2.2.2.2 Effect of Increasing p-Nitrophenol Loading Rates on the p-NP Removal Efficiencies in ABR Reactor. The effect of p-NP loading rate on the p-NP removal efficiencies in ABR was shown in Figure 4.25. The p-NP removal efficiency increased from 89% to 96% when the p-NP loading rate increased from 0.96 to 3.4 g/m^{3}day. After this p-NP loading rate, the p-NP removal efficiencies remained constant as 99%. This can be explained with the acclimation of methanogen *Archaea* bacteria to p-NP. p-NP removal efficiency was measured as 99% even at maximum p-NP loading rate. The effluent p-NP concentration was 7.25 mg/l at maximum p-NP loading rate of 67.9 g/m^{3}day. These results were compared with the results reported by Sponza & Kuşçu (2005). p-NP removal efficiency was around % 92 in AMBR reactor at a p-NP loading rate of 9.63 g/m^{3}day, while p-NP removal efficiency was found as 99% in ABR reactor at this loading rate. In another study, p-NP removal efficiency was found as 82% in a lab-scale digestor (Haghighi-Podeh, Bhattacharya, & Qu, (1995). Tseng & Lin, (1994) obtained a p-NP removal efficiency of 90% at a p-NP loading rate of 900 mg/l.day in an anaerobic biological fluidized-bed reactor system.

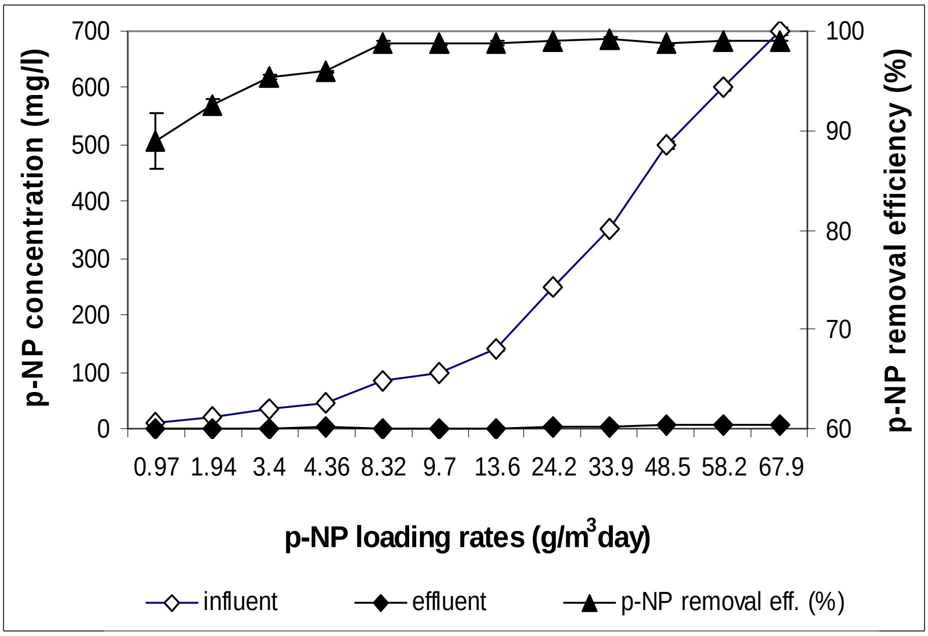


Figure 4.25 Effect of p-NP loading rate on COD removal efficiencies in ABR reactor.

4.2.2.2.3 Effect of Increasing p-NP Loading Rate on the Total and the Methane Gas Productions and Methane Percentages in ABR Reactor. The total and methane gas production rates and methane contents in ABR were shown in Figure 4.26. The daily total gas and methane gas production rates did not show a big change with increasing of p-NP loading rates. However, methane percentage decreased as the p-NP loading rate increased. The total gas and methane gas production rates were about 1000-1300 ml/day and 2000-2600 ml/day, respectively at p-NP loading rates varying between 0.97 and 67.9 g/m^{3}day. However, the methane percentage decreased from 54% to 42% as a p-NP loading rates increased from 0.97 to 67.9 g/m^{3}day. The high methane content was 54% at p-NP loading rates of 0.98 g/m^{3}day. Methane percentage was found as 42% at the maximum p-NP loading rate. Optimum total gas, methane gas and methane percentage were found as 2300 ml/day, 1300 ml/day and 50%, respectively, at p-NP loading rates varied between 0.98 g/m^{3}day and 8.32 g/m^{3}day.

Figure 4.27 shows the methane yields at increasing p-NP loading rates. The methane yield was 0.383 m^{3}CH$_4$/kg COD removed at p-NP loading rate of 0.97 g/m^{3}day. After this loading rate, methane yield began to decrease. The methane yield decreased to 0.239 m^{3}CH$_4$/kg COD removed at p-NP loading rate of 13.6 g/m^{3}day. The methane yield remained approximately 0.23 m^{3}CH$_4$/kg COD between p-NP loading rates of 13.6 and 33.9 g/m^{3}day. After that, methane yield increased to 0.25 m^{3}CH$_4$/kg COD removed at p-NP loading rates of 48.5 g/m^{3}day. Then the methane yield again decreased to 0.23 m^{3}CH$_4$/kg COD removed at p-NP loading rates of 58.2 g/m^{3}day. The methane yield was 0.21 m^{3}CH$_4$/kg COD removed at maximum p-NP loading rate of 67.9 g/m^{3}day. This shows that the methane *Archaea* bacteria acclimated to p-NP after a p-NP loading rate of 13.6 g/m^{3}day corresponding a p-NP concentration of 140 mg/l. After this loading rate, methane yield decreased to 0.79 m^{3}CH$_4$/kg COD removed. Generally, as the p-NP loading rates increased the methane yield decreased (R^2=0.85, df=11, F=27, p=0.0003).

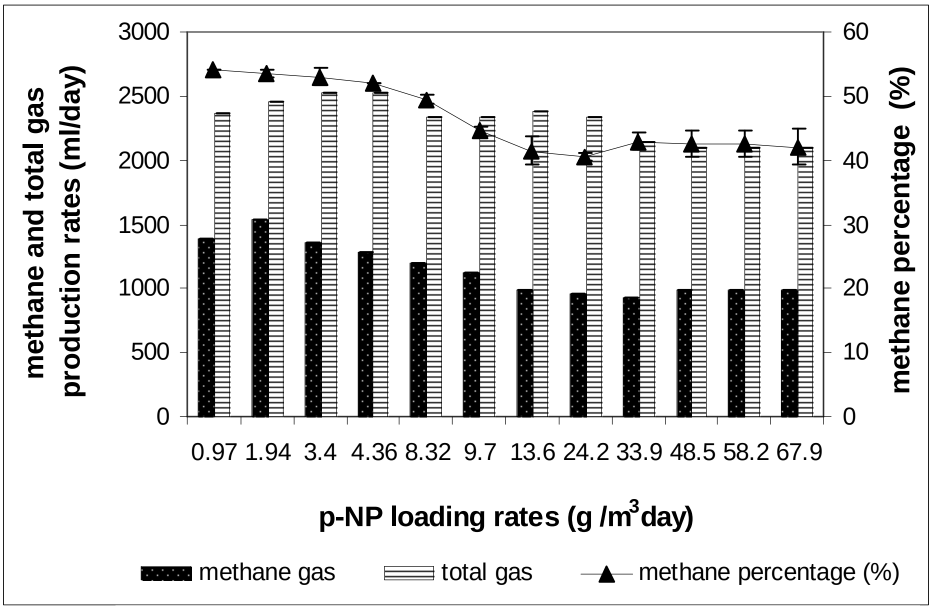


Figure 4.26 Total and methane gas production rates and methane percentage in ABR.

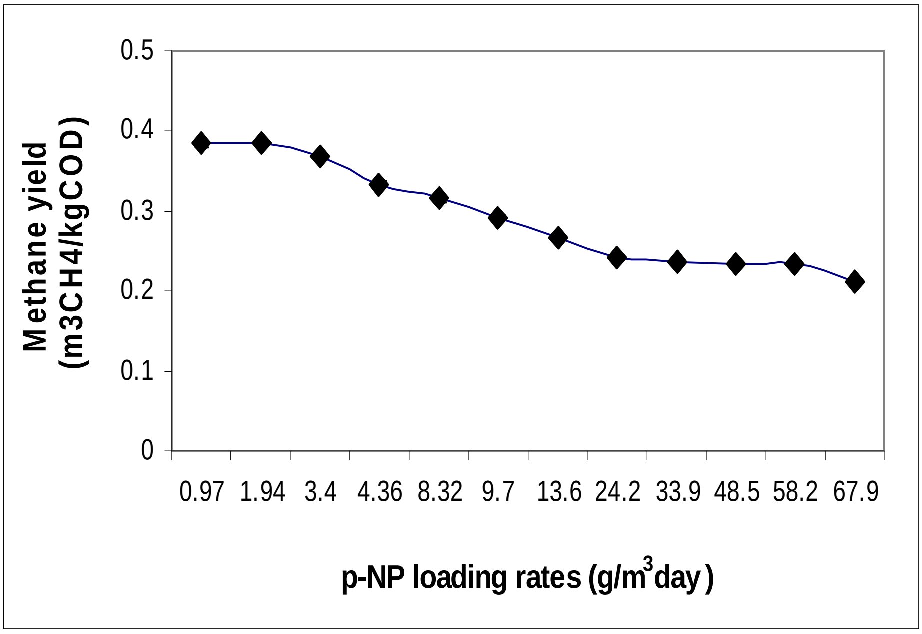


Figure 4.27 Methane yield at increasing p-NP loading rates in ABR.

4.2.2.2.4 Effect of Compartments in ABR Reactor on COD, p-NP removal Efficiencies and p-AP Production at Increasing p-NP Loading Rates. Figure 4.28 shows the variation of COD (a), p-NP (b) removal efficiencies and p-AP production (c) in compartments of the ABR reactor at increasing p-NP loading rates. The most of the influent COD and p-NP was removed in compartment 1 in ABR reactor. The COD removal efficiencies varied between 74% and 93% in this compartment. A small amount of COD removal occurred in the subsequent compartments. The COD removal efficiencies varied between 10–40% and 5–40% in compartments 2 and 3, respectively. This shows that acidogenesis takes place in compartment 1 while methanogenesis occurred in compartments 2 and 3. Uyanik, Sallis, & Anderson (2002) found that most of the influent COD was removed in first compartment of ABR (E=80%), with smaller removals occurring in compartment 2 (E=15%), and compartment 3 and 4 removing the remaining fraction of influent COD (E=5%). This result is similar with our removal efficiencies results.

Figure 4.28 (b) shows p-NP removal efficiencies in compartments of the ABR reactor at increasing p-NP loading rates. The p-NP removal efficiencies in the compartment 1 were higher than the other compartments (>90% p-NP removal). For example, p-NP concentration of 700 mg/l in influent decreased to 9.5 mg/l in compartment 1, to 8.4 mg/l in compartment 2 and to 7.25 mg/l in compartment 3. The removal efficiencies in compartments 2 and 3 varied between 5% and 50% depending to low COD and p-NP concentrations remaining from the compartment 1 through anaerobic treatment. As shown in Figure 4.28 (c), p-AP concentration in compartments increased with increasing the p-NP loading rate. p-AP concentration increased from 5 mg/l to 342 mg/l in compartment 1, from 1.5 mg/l to 316 mg/l in compartment 2, from 2 mg/l to 294 mg/l in compartment 3, when the p-NP loading rate increased from 0.97 to 67.9 g/m^{3}day. p-AP values in compartment 1 were higher than the other two compartments. The most of p-AP produced in the first compartment. Melgoza & Buitron, (2001) investigated the kinetic of p-NP transformation under anaerobic condition. Melgoza & Buitron, (2001) found that the majority of the p-NP transformation occurs in the first 4 hours of anaerobic phase, but maximum production of p-AP is reached after 8 hours. The p-AP degradation is very fast compared to their formation, since this intermetabolitt is degraded in the first 2.5 hours. These results explain why the majority of p-AP produced in the first compartment of ABR.

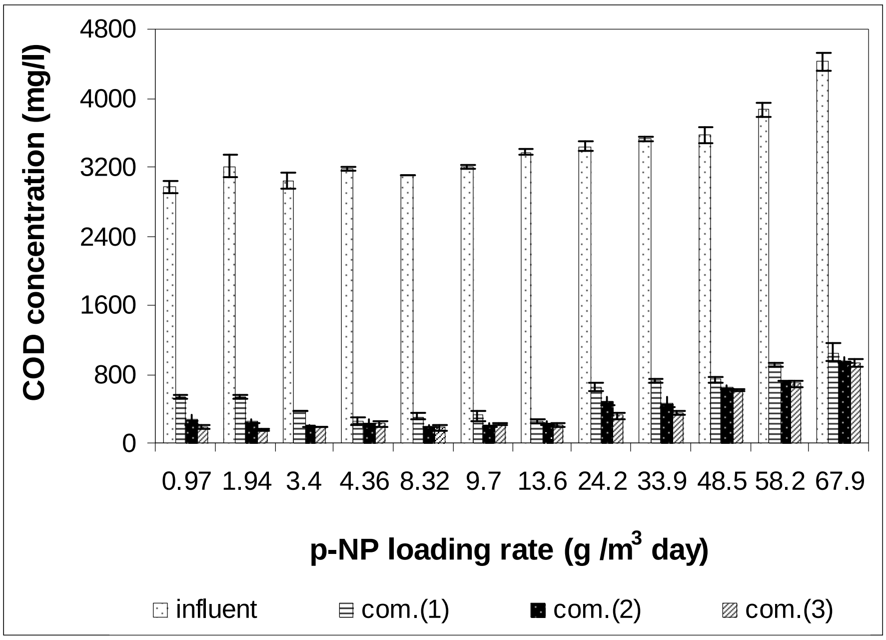


Figure 4.28 The variations of COD removal efficiencies in compartments of the ABR reactor (a).

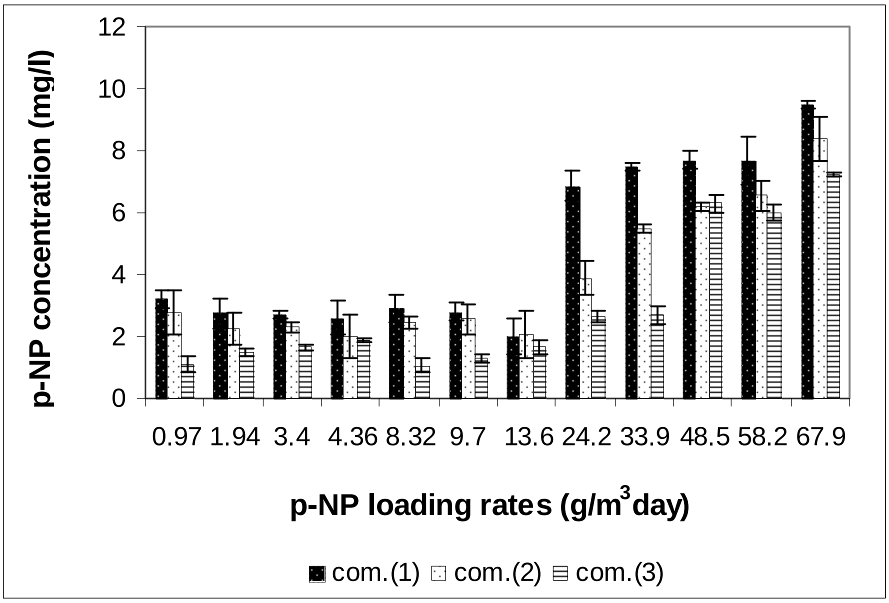


Figure 4.28 The variations of p-NP removal efficiencies in compartments of the ABR reactor (b).

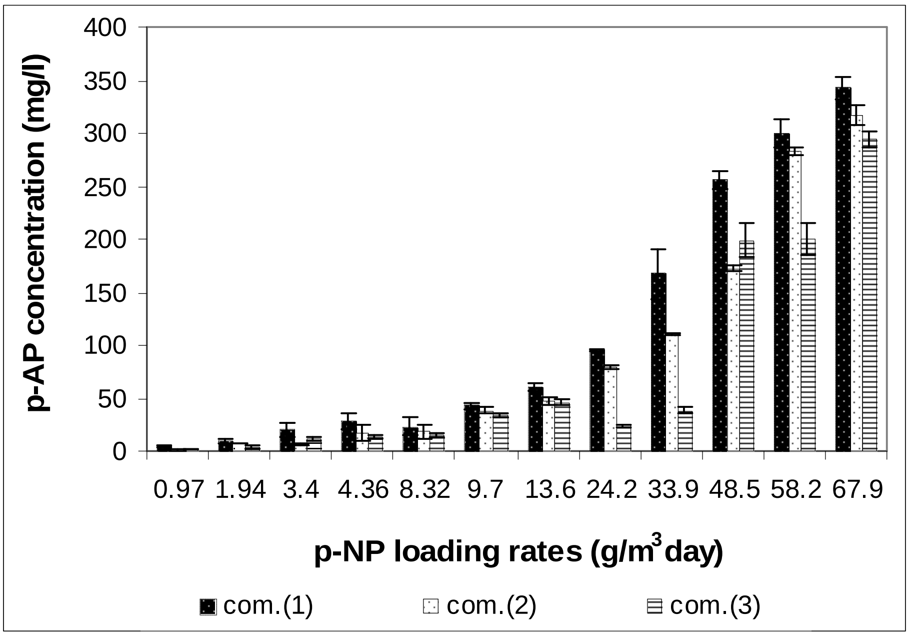


Figure 4.28 The variations of p-AP production in compartments of the ABR (c).

4.2.2.2.5 The Variations of pH, TVFA, Bicarbonate Alkalinity (Bic.Alk.) and TVFA/Bic.Alk. ratio in Compartments of the ABR Reactor at Increasing p-NP Loading Rates. Figure 4.29 shows the pH, TVFA, bicarbonate alkalinity and TVFA/Bic.Alk. ratio variations in ABR reactor at increasing p-NP loading rates. As shown in Fig. 4.29 (a), the pH values in the first compartment was lower than the other compartments. pH values in the first compartment varied between 7.1 and 7.5 at all p-NP loading rates. The low pH in initial compartment was since acetogenesis take place and TVFA accumulated in the compartment. pH values increased in compartments 1 and 2. pH varied between 7.2 and 7.6 in the second compartment and between 7.3 and 7.6 in the third compartment. This shows that the pH values in compartments were between optimum pH values (pH 6.5 and 8.3) suggested for anaerobic degradation (Speece, 1996).

TVFA concentrations were high in the first compartment compared to the other compartments (See 4.29. (b)). TVFA concentrations increased from 55 mg/l to 112 mg/l as p-NP loading rate increased from 0.97 to 67.9 g/m^{3}day in first compartment. TVFA concentrations decreased in compartments 2 and 3. TVFA concentrations were zero until a p-NP loading rate of 24.2 g/m^{3}day in compartment 2 and then the TVFA concentration increased from 78 mg/l to 87 mg/l with increasing p-NP loading rate from 24.2 to 67.9 g/m^{3}day. TVFA concentrations were zero in compartment 3 at all p-NP loading rates. Low pH and higher TVFA in compartment 1 illustrate the horizontal separation of acidogenesis and methanogenesis through ABR reactor (Bell, 2002). Compartment 2 and 3 were not affected from the p-NP loading rates which illustrates the ability of the compartmentalize reactor in order to protect the sensitive methanogenesis.

Bic.Alk. concentration was approximately 3000-3200 mg/l in the influent. Bic.Alk. concentration decreased in compartment 1 (See Fig. 4.29 (c)). The bicarbonate alkalinity concentrations varied between 2900 and 3200 mg/l in compartment 1. This indicates the utilization of alkalinity to buffer the VFA and CO_2 produced from the anaerobic co-metabolism of p-NP. Bic.Alk. concentration increased in compartments 2 and 3, with the exception of p-NP loading rate of 67.9 g/m3day since high TVFA accumulated in the compartment 2. Fig. 4.29 (d) shows TVFA/Bic.Alk. ratios in compartments. TVFA/Bic.Alk. ratios varied between 0.01 and 0.03 at compartment 1, between 0.02 and 0.03 in compartment 2 at increasing p-NP concentration. TVFA/Bic.Alk. ratios found as zero in compartment 3 at all p-NP loading rates. TVFA/Bic.Alk. ratios in the effluent and compartments of the ABR were lower than 0.4. These results indicated the stability of ABR reactor as reported by Behling et al., (1997).

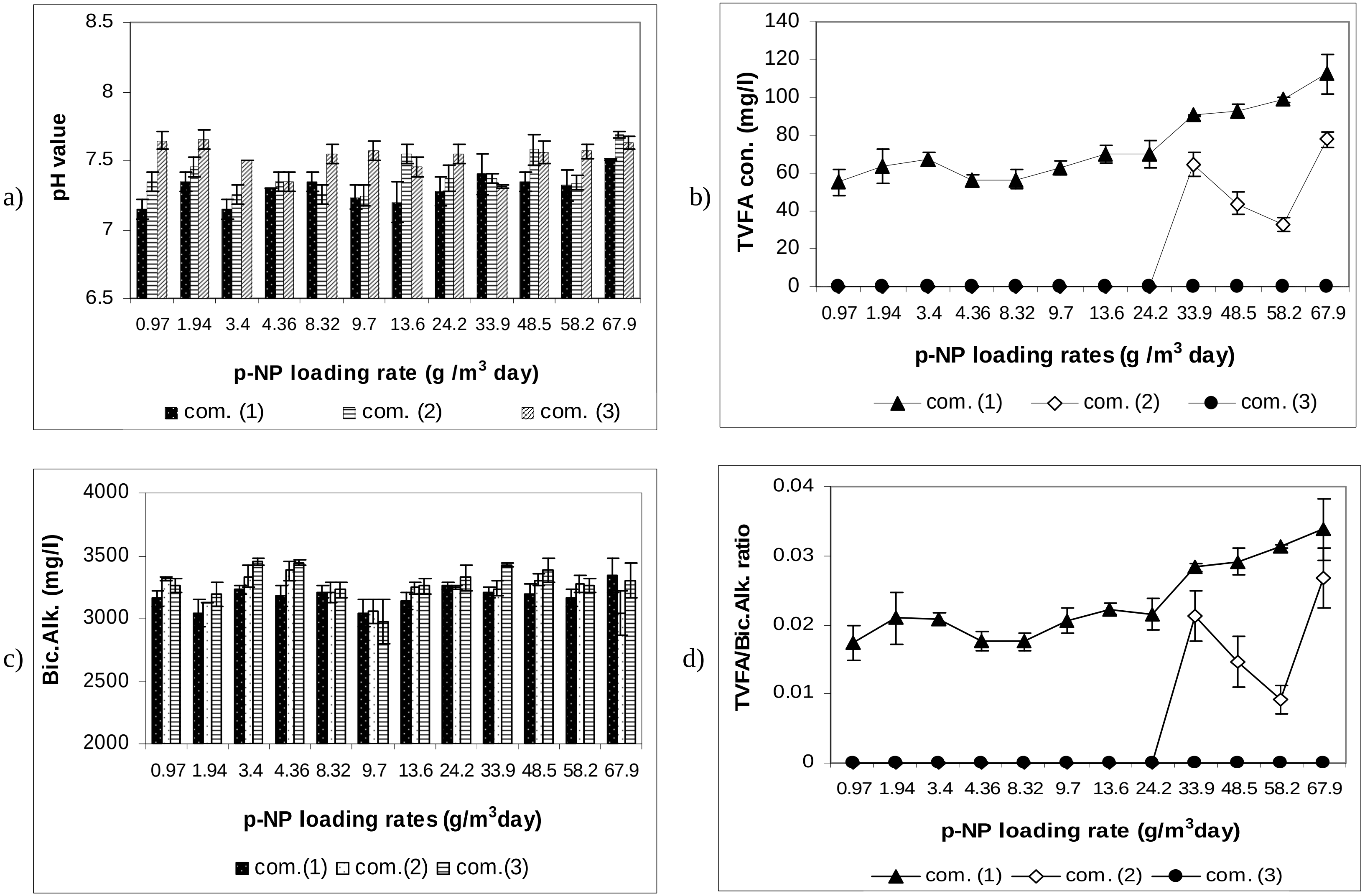


Figure 4.29 The variations of pH (a), TVFA (b), Bic.Alk. (c) and TVFA/Bic.Alk. ratio (d) in ABR at increasing p-NP loading rates.

4.2.2.2.6 Performance of Sequential Anaerobic (ABR)/Aerobic (CSTR) Reactor System. Figure 4.30 shows the overall COD and p-NP removal efficiencies in sequential ABR/CSTR reactor system. The COD removal efficiencies were up to 90% until a p-NP loading rate of 48.5 g/m^3.day in sequential ABR/CSTR reactor system. The p-NP removal efficiencies increased from 97 % to 99% as p-NP loading rates increased from 0.97 to 8.32 g/m^{3}day in sequential ABR/CSTR reactor system. After this p-NP loading rate, the p-NP removal efficiencies remained constant 99% at all p-NP loading rates. This indicates the performance of ABR reactor since 99% of p-NP was transformed to p-AP in ABR reactor (See Fig. 4.25). Residual p-NP from ABR reactor removed in aerobic reactor (only 1-4% of total p-NP efficiency). p-AP was mineralized to CO_2, H_2O in aerobic phase. Figure 4.31 shows the p-AP removal efficiencies in aerobic phase. Approximately 100% mineralization of p-AP was observed until a p-NP loading of 8.32 g/m^{3}day in aerobic phase. Then p-AP removal efficiency decreased from 100% to 80%. This could be explained by the p-NP concentration in the influent of CSTR showing toxic effect on aerobic bacteria. p-AP removal efficiency was approximately 80% at p-NP loading rates between 24.2 and 33.9 g/m^{3}day. After these loading rates, p-AP removal efficiencies began rising. p-AP removal efficiencies increased from 80% to 95% at a p-NP loading rate of 48.5 g/m^{3}day. p-AP removal efficiency was 90% at maximum p-NP loading rate of 67.9 p-AP g/m^{3}day. This can be explained with the acclimation of aerobic microorganism to p-NP. Figure 4.32 shows p-NP concentration in the influent and effluent of aerobic CSTR reactor. p-NP concentration began increase after a p-NP loading rate of 8.32 g/m^{3}day in the influent of CSTR reactor (anaerobic reactor effluent). p-NP concentration in the influent of CSTR increased from 1 mg/l to 7.25 mg/l as the p-NP loading rates increased from 8.32 g/m^{3}day to 67.9 g/m^3.day. p-NP removal efficiencies were found as 40-60 % in CSTR reactor at all p-NP loading rates. p-NP concentration in the effluent of CSTR increased from 1 mg/l to 2.25 mg/l as p-NP loading rates increased from 8.32 g/m^{3}day to 67.9 g/m^{3}day.

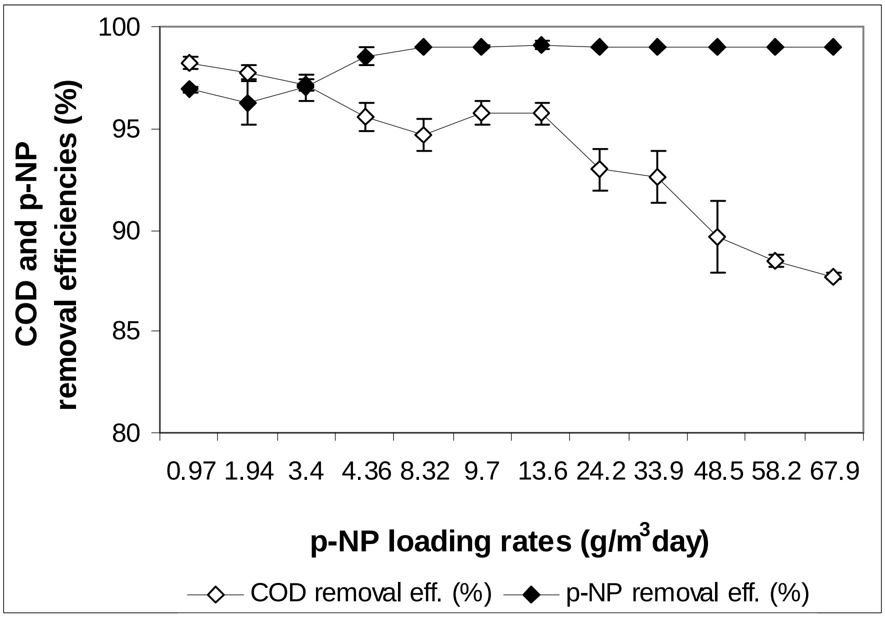


Figure 4.30 The overall COD and p-NP removal efficiencies in sequential ABR/CSTR reactor system.

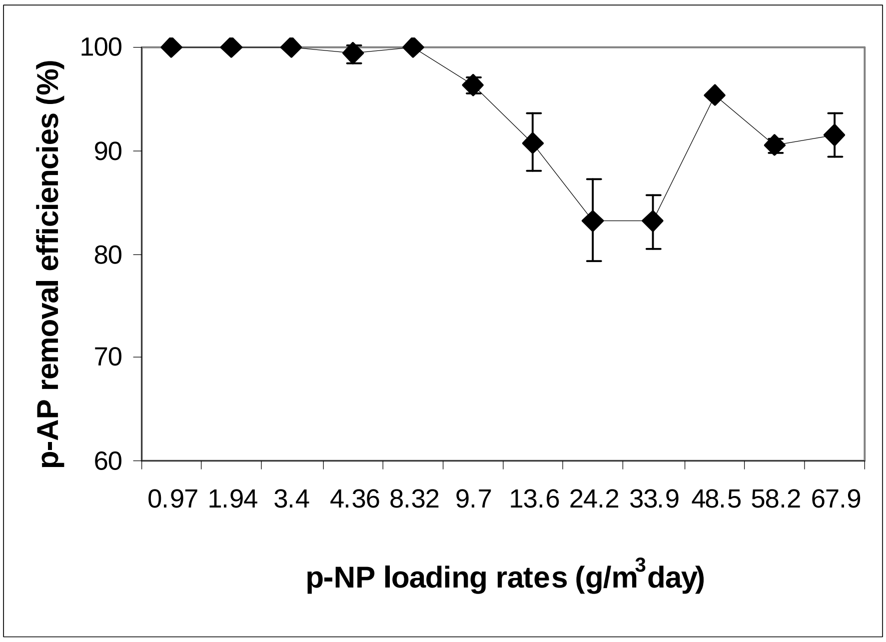


Figure 4.31 p-AP removal efficiencies in aerobic CSTR reactor.

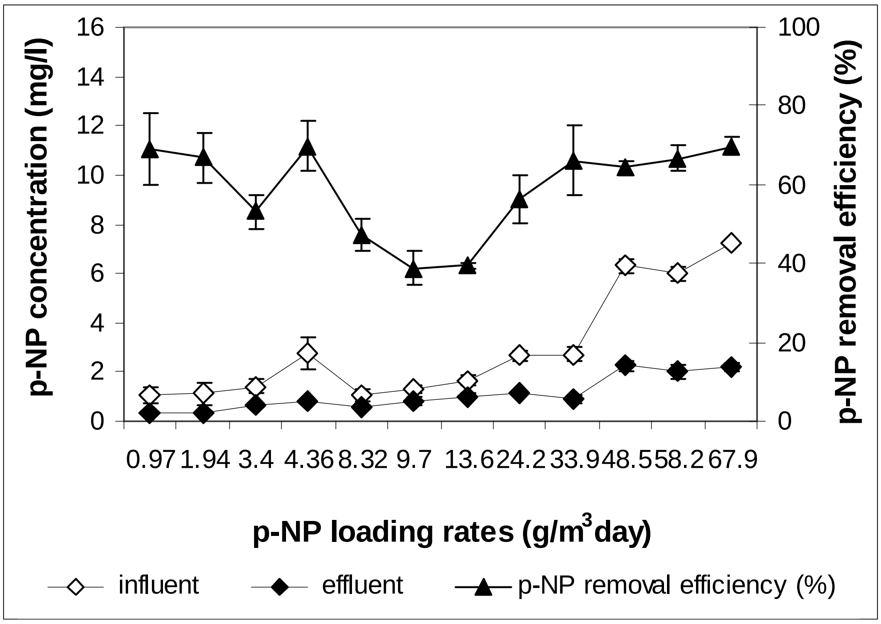


Figure 4.32 p-NP concentrations in the influent and effluent of CSTR reactor and p-NP removal efficiencies at increasing p-NP concentration.

4.2.2.3 Effect of Hydraulic Retention Time (HRT) on Performance of ABR Reactor

4.2.2.3.1 Effect of HRTs on COD and p-NP Removal Efficiencies in Anaerobic ABR Reactor. The effect of hydraulic retention times (HRTs) on the COD (a) and p-NP (b) removal efficiencies was shown in Figure 4.33. The influent p-NP concentration was kept constant as 85 mg/l. As shown in Fig. 4.33 (a), the influent COD concentration was approximately 3100-3200 mg/l since 85 mg/l p-NP gives an additional COD concentration to total COD through continuous operation. 85 mg/l of p-NP gave approximately COD of 120-150 mg/l. 99% COD removal efficiency was obtained at a HRT of 10.38 days in ABR reactor. The effluent COD concentration was 25 mg/l at this HRT. As HRT was decreased, there was a slight increase in the COD concentration in the effluent. The COD removal efficiencies decreased as the HRT decreased (R^2=0.97, df=5, F=59.7, p=0.001).When the HRT were decreased from 5.19 days (OLR= 0.62 kg COD/m^3 day) to 1 day (OLR=3.16 kg COD/m^3 day), the effluent COD concentration increased from 92 mg/l to 610 mg/l. COD removal efficiency also decreased from 97% to 71%, respectively (R^2=0.97, df=5, F=71.2, p=0.001). The COD removal efficiency exhibited a good performance (E>95%) until a HRT of 3.4 days in ABR. Akunna & Clark, (2000) investigated the performance of anaerobic baffled reactor treated a whisky distillery wastewater at four HRTs (10, 7, 4 and 2 days). The maximum COD removal efficiency was observed at a HRT of 4 days (E=93%). Kuscu & Sponza, (2006) reported that maximum COD removal efficiencies (E=90–92%) were observed in AMBR reactor at HRTs varying between 3.4 and 10.38 days.

The effect of HRT on the p-NP removal efficiencies are shown in Fig. 4.33 (b). 98% p-NP removal efficiency was obtained at a HRT of 10.38 days. The optimum p-NP removal efficiencies (E=95-98) were observed at HRTs varied between 10.38 days and 1.5 days. Maximum p-NP removal efficiency was 98% at HRT of 10.38 days days, giving maximum COD removal efficiency (E=99%). The effluent p-NP concentration was measured between 1.8 and 2.7 mg/l at these HRTs. p-NP removal efficiency was 90% at minimum HRT of 1 day. The effluent concentration was found as 7.3 mg/l at this HRT. However ABR reactor exhibited high p-NP removal efficiency even at loves HRT or at high OLRs. ABR showed a good performance even at shock OLRs. Uyanik, Sallis, & Anderson, (2002) investigated the performance of an anaerobic baffled reactor (ABR) treating an ice-cream wastewater at HRTs varied between 0.43 and 10 days. In this study, COD removal efficiency was 99% at all HRTs. A great part of COD (approximately 80%) was removed in the first compartment of ABR. High efficiency came from its compartmentalised structure of this reactor.

As seen in Figs. 4.33 (a)(b) the COD removal efficiency decreased with decreasing HRT, while no variation was observed in p-NP removal. This result could be explained by the biodegradation of the p-NP to intermediate products such as aromatic amines (p-AP) and (phenol) under reductive anaerobic conditions, which not ultimately metabolized and caused to residual COD. In other words, the p-NP was converted to p-AP under anaerobic conditions and this p-AP was measured as COD.

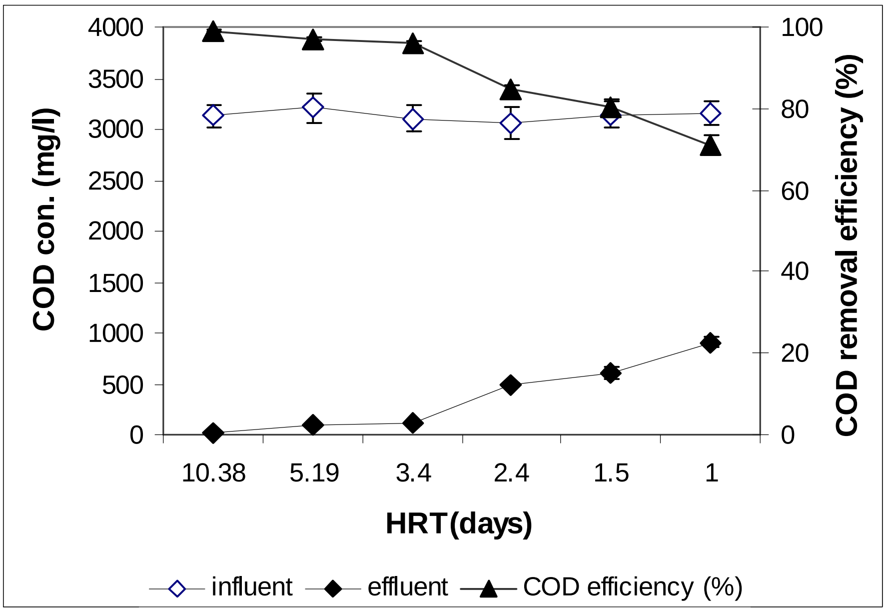


Figure 4.33 The effect of HRTs on COD removal efficiencies in ABR (a).

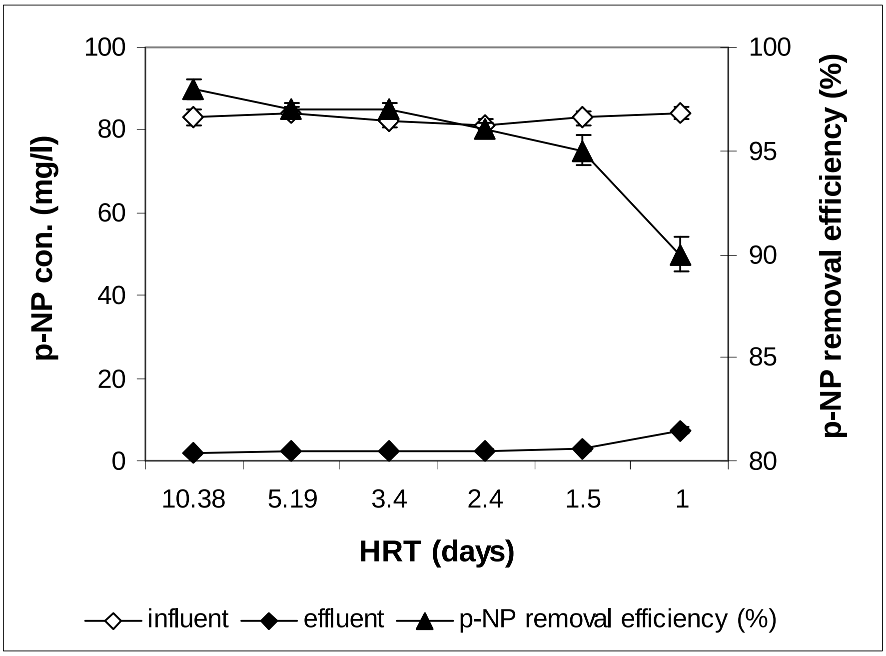


Figure 4.33 The effect of HRTs on p-NP removal efficiencies in ABR (b).

4.2.2.3.2 Effect of HRTs on Gas Productions and Methane Percentage in Anaerobic ABR Reactor. Biogas production was monitored through the operation of the ABR reactor, particularly for detection the methanogenic activity. From Fig. 4.34 it can be seen that the total gas and methane gas production rates increased from 2.2 to 17.7 l/day and from 0.9 to 4.3 l/day, respectively as the HRT decreased from 10.38 days to 1 day as the OLR increased from 0.3 to 3.16 kg COD/m^3 day, respectively. Total gas and methane gas production rates increased with decreasing HRTs (R^2=0.87, df=5, F=12.5, p=0.02). But methane gas production rates decreased from 5.12 to 4.32 l/day at a HRT of 1 day. The methane percentages of the biogas were approximately 38-39% until a HRT of 3.4 days. After that HRT, the methane content of biogas decreased from 39% to 25% as the HRT decreased from 3.4 days to 1 day. Maximum methane gas production (4.4 l/day) was obtained at 1.5 day of HRT. A strong linear correlation between COD removal and methane percentage, COD removal and methane gas production was observed (R^2=0.94; d.f=5, F=35.51, p = 0.03 and R^2=0.85, d.f =5, F =11.28, p = 0.01). H_2S gas was monitored through continuous operation in order to determine the composition of the total gas since the synthetic media used in this study contained organic sulfured substances. It was observed that sulfate-reducing bacteria can outcompete with methanogens for glucose-COD since hydrogen sulfide production can be predominant over methane gas production at high OLRs (Speece, 1996). Furthermore, acetate could be converted to H_2S instead of methane at high OLRs. In this study, H_2S concentrations in the gas was measured between 160 and 195 ppm at organic loading rates as high as 2.1 and 3.16 kg COD/m^3 day. At low OLRs no H_2S production was observed since methanogenesis was predominant. Since the anaerobic treatment of a wastewater is directly related to the amount of methane produced, the amount of methane generated per kilogram of COD stabilized should be taken as an indicator for determining the stabilization degree of p-NP and COD. Fig. 4.35 shows the variations of methane yields versus increasing OLRs. It was observed that the methane yield decreased partly with decreasing HRTs. The methane yield was 0.16 m^3 CH_4/kg COD removed at HRT of 10.38 days. The methane yield decreased from 0.16 to 0.07 m^3 CH_4/kg COD removed as HRT decreased from 10.38 days to 1 day. Uyanik, Sallis, & Andersan (2002) found that the methane yield was 0.15 m^3 CH_4/kg

COD at an OLR of 0.62 kg COD/m^{3}day in an anaerobic baffled reactor (ABR) treating ice-cream wastewater. Sponza & Kuşçu (2005) found that the methane production rate decreased from 0.34 to 0.28 m^3 methane/kg COD, when the p- NP loading rates increased from 9.7 to 67.9 g/m^{3}day in ABR reactor. This shows that the methane yield affected significantly from the organic loading rates (HRTs) containing p-NP.

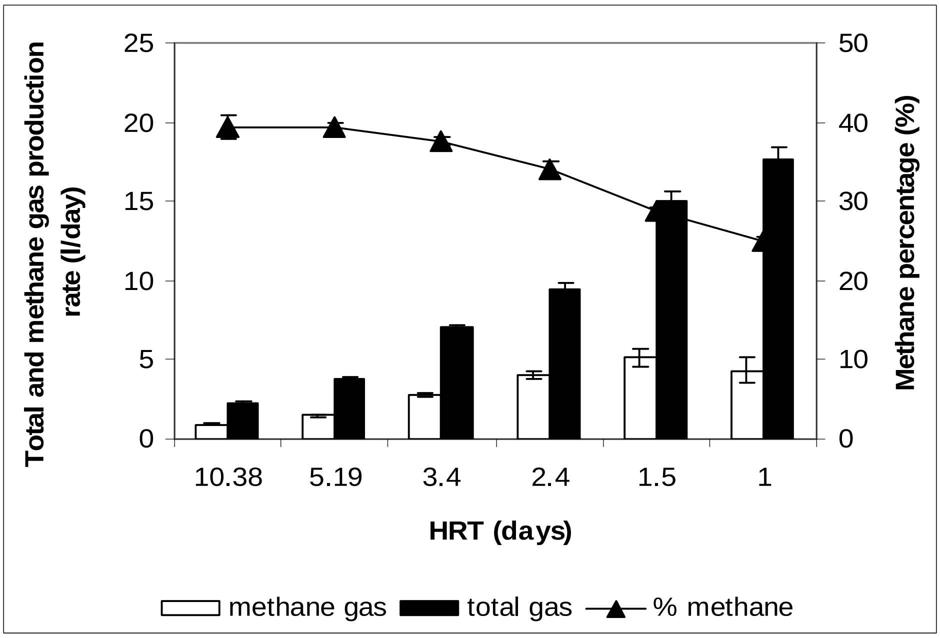


Figure 4.34 Total gas, methane gas productions and methane content in ABR at different HRTs.

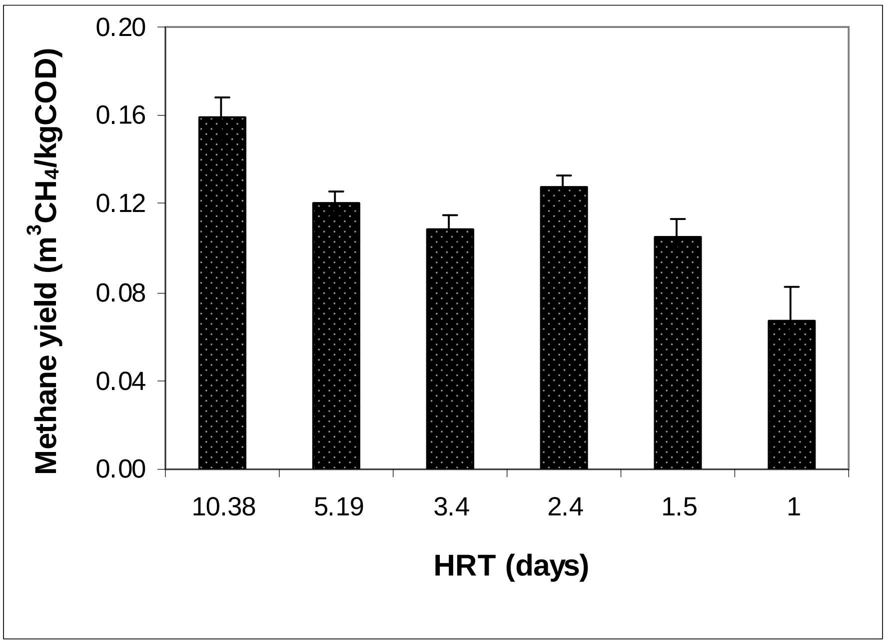


Figure 4.35 Methane yields in ABR reactor at different HRTs.

4.2.2.3.3 Effects of HRTs on pH, Total Volatile Fatty Acid (TVFA), Bicarbonate Alkalinity (Bic.Alk.) and TVFA/Bic.Alk. Ratio Variations in ABR Reactor. Figure 4.36 shows the pH, TVFA, Bic.Alk. and TVFA/Bic.Alk. ratio in the effluent and compartments of ABR at different HRTs. The pH values in compartment 1 were lower than other compartments. This caused high TVFA concentration in the first compartment. However the pH values in the effluent and compartments of ABR varied between 6.8 and 7.8, which is between optimum values for anaerobic treatment. As shown in Fig. 4.36 (a), the pH values in the first compartment were lower than the other two compartments. The possible reason for this decrease in pH in the first compartment can be explained by the increases of TVFA levels (See Fig. 4.36 (b)). The highest VFA concentration was found in the first compartment, which then decreased in compartments 2 and 3 at all HRTs. The all VFAs could be removed in ABR until a HRT of 2.4 days. After this HRT, TVFA concentration in the effluent increased and was measured as 560 mg/l at HRT of 1 day. Under these conditions the acetate and propionic acid concentrations increased to 350–400 mg/l (data not shown). At high OLRs and low HRTs the strength of toxicity of wastewater and intermediate products (p-aminophenol, phenol) caused preacidification resulting in accumulation of COD (Lettinga et al., 1984). The COD did not convert to methane, resulting in an accumulation of VFA. At high HRT the acetate and propionate concentrations decreased to 40–60 mg/l (data not shown). From Fig. 4.36 (c), it can be seen that Bic.Alk. concentrations in compartments decreased from 3500 to 1820 mg/l since the HRT were decreased. In anaerobic reactor system TVFA/Bic.Alk. ratio gives necessary information to determine the stability of the anaerobic reactor. If the TVFA/Bic.Alk. ratio is lower than 0.4, the reactor is stable. When the TVFA/Bic.Alk. ratio is lower than 0.8, the reactor system is moderately stable or unstable (Behling et al., 1997). As shown in Fig. 4.36 (d). This ratio varied between 0.02 and 0.53 in compartment 1. The TVFA/Bic.Alk. ratio was between 0.01 and 0.52 in compartment 2 while this ratio varied between 0.0 and 0.27 in compartment 3 as the HRTs were decreased from 10.38 days to 1 day. ABR reactor was stable as repoted by Behling et al., (1997) since the TVFA/Bic.Alk. ratios in the effluent were lower than 0.4.

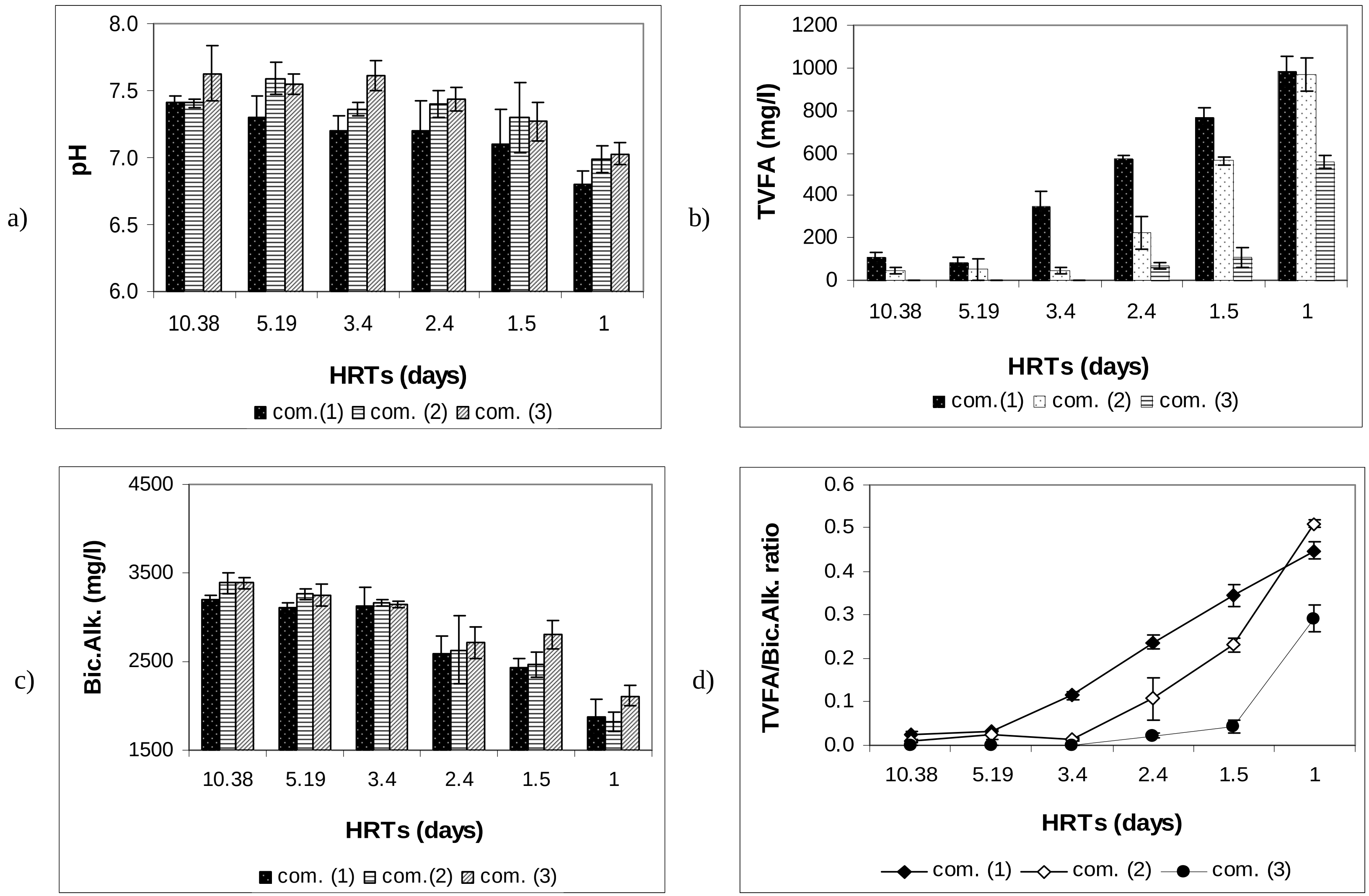


Figure 4.36 The variations of pH, TVFA, Bic.Alk. and TVFA/Bic. Alk. ratio in the compartments of ABR at different HRTs.

4.2.2.3.4 The Effect of HRT on Variations of p-Aminophenol (p-AP) and Phenol Production in ABR. Figure 4.37 shows the variation of p-AP and phenol in the effluent of ABR with decreasing HRTs. In the present study relatively high levels of p-AP and phenol were observed at low HRTs. As shown in Fig. 4.37 p-AP concentration increased from 10 mg/l to 35 mg/l as the HRT decreased from 10.38 days to 1 day. Phenol concentrations in the effluent of ABR did not varied with decreasing HRT. Phenol concentration was measured approximately between 4.5 and 7 mg/l as the HRT decreased from 10.38 to 1 day.

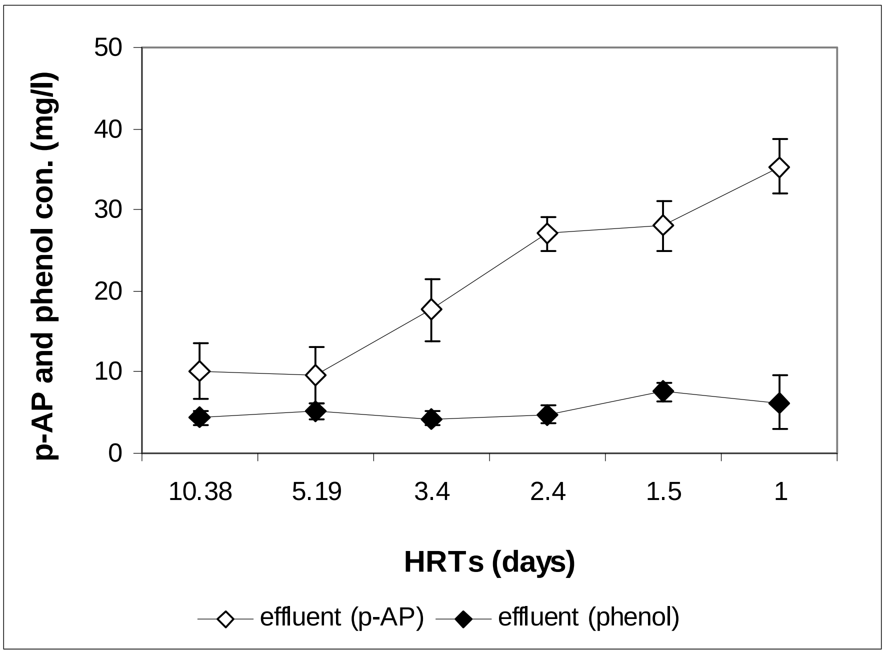


Figure 4.37 The variations of p-AP and phenol concentrations in effluent of the ABR at different HRTs.

4.2.2.3.5 Effect of Compartments of ABR on COD, p-NP Removal Efficiencies at Different HRTs. Figure 4.38 shows the effect of compartmentalisation on COD and p-NP removal efficiencies at different HRTs. As indicated in Figure 4.38 (a), the most of the influent COD was removed in compartment 1 (E=50%-97%). The COD removal efficiencies were approximately 97% at high HRT such as 10.38 and 5.19 days. The COD concentration decreased from 3184 to 74 mg/l in compartment 1 at a HRT of 10.38 days (E=97%). The smaller COD removals (E=17 %-37%) occurred in compartment 2 and the remaining fraction of influent COD was removed with removal efficiencies varying between 14 % and 28 % in compartment 3. Figure 4.38 (b), shows the variations of p-NP in compartments of AMBR. The p-NP concentration increased from 4.9 to 43 mg/l in the initial compartment, from 4.7 to 13 mg/l in the second compartment, and from 1.8 to 7.3 mg/l in the third compartment as HRT decreased from 10.38 to 1 day. p-NP removal efficiencies were approximately 85-93% in the initial compartment, 28-40 % in the second compartment and 30-40 % in the third compartment when the HRTs were decreased from 10.38 to 1 day.

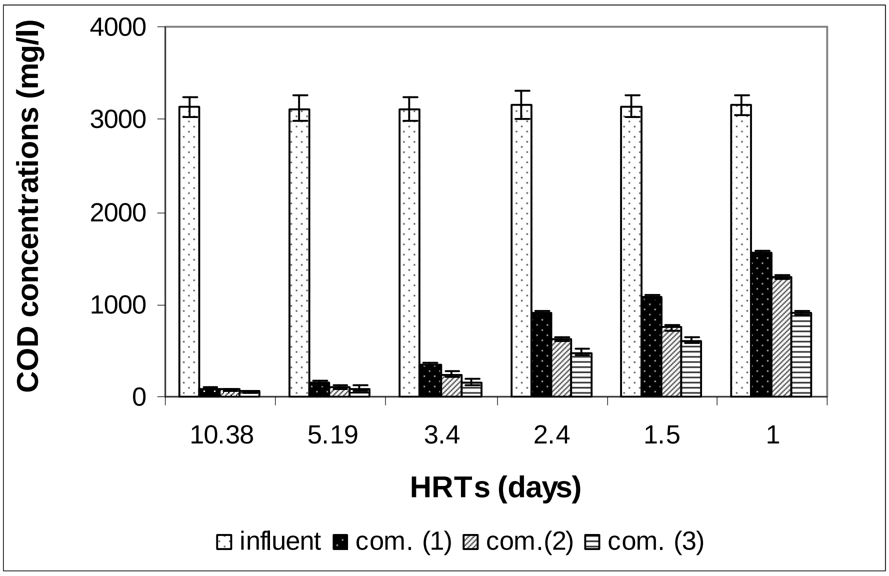


Figure 4.38 The effect of compartments on COD removal efficiencies in ABR at different HRTs (a).

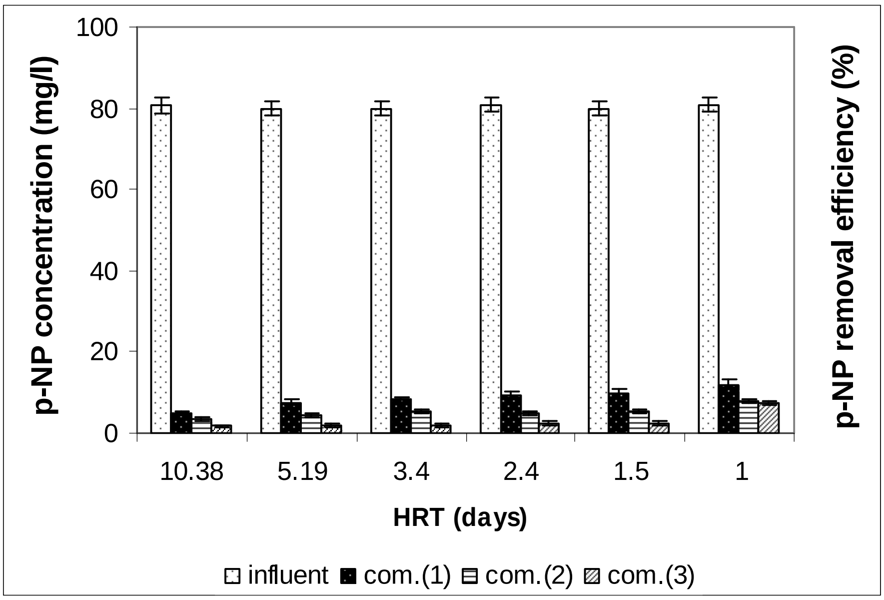


Figure 4.38 The effect of compartmentalisation on p-NP removal efficiencies in ABR at different HRTs (b).

4.2.2.3.6 Effect of Compartments of ABR on p-AP and Phenol Production at Different HRTs. Figure 4.39 shows the effect of compartments on p-AP (a) and phenol (b) production at different HRTs. As shown in the Figure 4.39 (a) and (b), p-AP and phenol concentration were higher in the first compartment than the other compartment at high HRTs sush as 5.19 and 10.38 days. At a HRT of 10.38 days, the p-AP and phenol concentrations were found as 18 mg/l and 16 mg/l in the first compartment, 16 mg/l and 5 mg/l in the second compartment and 14 and 4.6 mg/l in the third compartment, respectively. This shows that the p-AP was transformed in compartment 1 and converted to phenol. Therefore the phenol is higher in the first compartment as the HRT decreased from 10.38 days to 3.4 days. However, phenol concentration is higher in the second and third compartments at lower HRT such as 2.4, 1.5 and 1 day. Melgoza & Buitron, (2001) found that the p-AP transformation rate was 14 mg p-AP/ gVSS-h. Transformation of p-AP to phenol was observed in compartment 2 and 3 at low HRTs. Phenol production was approximately 5 mg/l in whole ABR at HRT of 1 day.

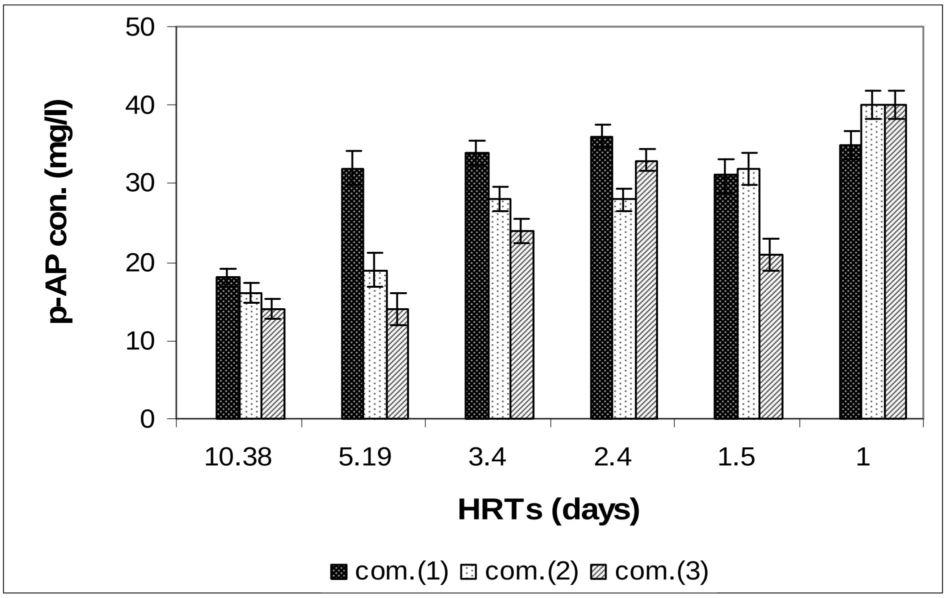


Figure 4.39 Effect of compartments on p-AP production in ABR at different HRTs (a).

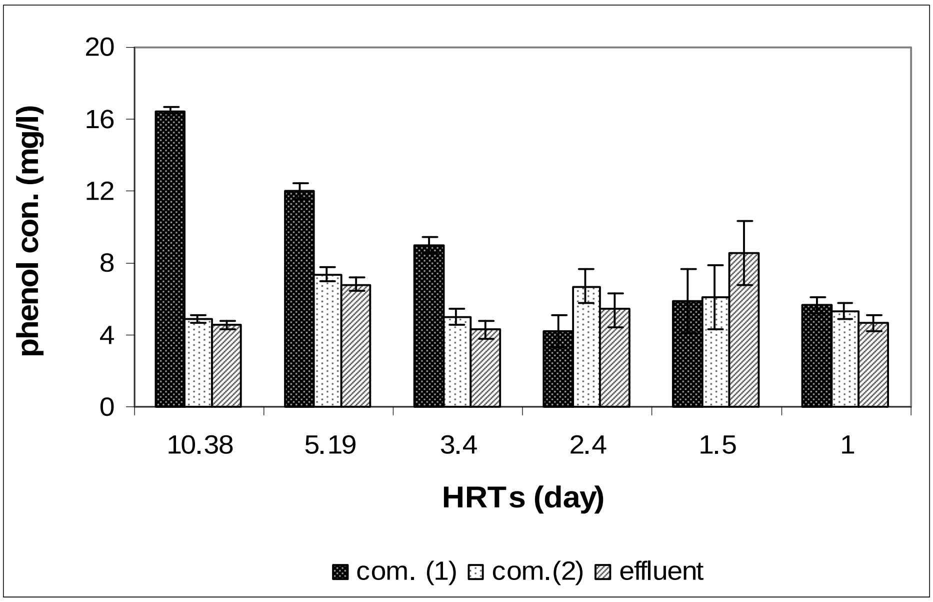


Figure 4.39 Effect of compartments on phenol production in ABR at different HRTs (b).

4.2.2.3.7 Total Removal Efficiencies in Sequential Anaerobic ABR/Aerobic CSTR Reactor System. Figure 4.40 shows the overall COD and p-NP removal efficiencies of anaerobic/aerobic sequential reactor system. The overall COD removal efficiencies in sequential anaerobic ABR/aerobic CSTR reactor system were up to 94% until a HRT of 2 days. The p-NP removal efficiency in ABR/CSTR reactor system varied between 93% and 99% at all HRTs. The COD and p-NP removal efficiencies decreased from 99% and 86% and from 99% to 93%, respectively when the HRT were decreased from 13.6 to 1.3 day. The optimum COD and p-NP removal efficiencies were found as 99% and 98%, respectively, at p-NP loading rate of 6.8 g/m^3day. As shown in Figure 4.38., the p-NP removal efficiencies did not show a significant variation with decreasing HRTs. This can be explained by the utilization of p-NP by the acetogens and methanogens together with glucose-COD while glucose-COD was not completely converted to methane at low HRTs. A low part of COD converted to p-AP and phenol were measured as COD in anaerobic reactor. The COD and p-NP removal efficiency in overall reactor system was 86% and 93%, respectively, at the minimum HRT of 1 day. 93–99% of p-NP mineralized to p-AP in anaerobic reactor into overall reactor at all HRTs. A degradation of approximately 95–100% of p-AP was found in the aerobic phase at HRTs varying between 2.4 and 10.38 days (See Table 4.6). The p-AP removal efficiency was 100% until a HRT of 5.19 days in aerobic reactor. p-AP removal efficiency was 58% at minimum HRT. Table 4.6 presents the nitrogen balance through p-NP degradation in anaerobic/aerobic sequential reactor system. A degradation of approximately 95–100% of p-AP was metabolized in the aerobic phase at HRTs varying between 10.38 days and 1 day (See Table 4.6). The p-AP removal efficiency was 100% and the effluent p-AP concentrations were found as zero until a HRT of 3.4 days in aerobic reactor. After this HRT, p-AP removal efficiency decreased from 100% to 58% and effluent p-AP concentrations increased from 0 mg/l to 16 mg/l in aerobic reactor (See Table 4.6). Phenol in the effluent of anaerobic reactor was removed with efficiencies varying between 28% and 38% in aerobic reactor. When HRT decreased, phenol concentration in the anaerobic effluent increased from 4.4 to 6.2 mg/l (See Table 4.6). p-AP, phenol and NH_4-N were anaerobic degradation products of p-NP which were transformed to end products by aerobic reactor. The generation of ammonia (NH_4-N) was observed through the anaerobic degradation of p-AP in ABR. The NH_4-N produced in the anaerobic phase converted to nitrite (NO_2) and nitrate (NO_3) as end products in the aerobic phase. It was observed that 42–97% of ammonia oxidized to nitrite and nitrate in aerobic phase. As shown in Table 4.6, a high amount of NH_4-N produced by the anaerobic bacteria was removed by the aerobic bacteria with removal efficiencies varied between 42% and 97%.

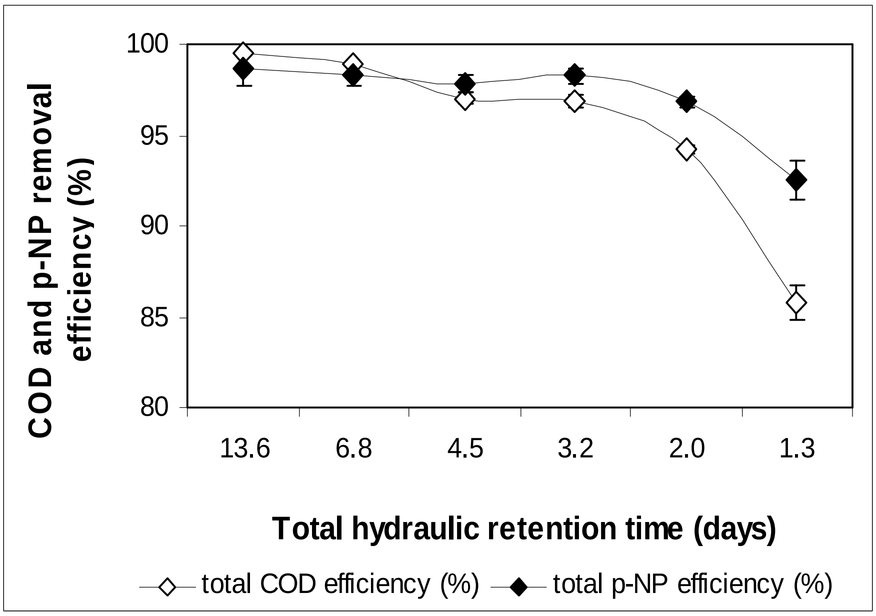


Figure 4.40 The overall COD and p-NP removal efficiencies in anaerobic (ABR)/aerobic (CSTR) sequential reactor system.

Table 4.6 The effluent p-NP, p-AP and phenol concentrations and nitrogen balance in anaerobic (ABR)/aerobic (CSTR) sequential reactor system.

HRT (day)	**p-nitrophenol (mg/l)**		**p-aminophenol (mg/l)**		**Phenol (mg/l)**		**NH_4-N (mg/l)**		**NO_2-N (mg/l)**		**NO_3-N (mg/l)**	
	Anaerobic effluent	Aerobic effluent	Anaerobic effluent	Aerobic effluent	Anaerobic effluent	Aerobic effluent	Anaerobic effluent	Aerobic effluent	Anaerobic effluent	Aerobic effluent	Anaerobic effluent	Aerobic effluent
10.38	1.8±0.4	1.1±0.7	10±3.5	0±0	4.4±0.8	3.2±0.3	133±14.7	4±0.7	0.1±0.06	4.9±1.3	0.6±0.2	163±15
5.19	2.2±0.3	1.4±0.4	9.6±3.9	0±0	5.2±1.0	3.7±0.8	131±11.1	8.5±4.3	0.1±0.06	3.1±1.6	1.1±0.5	74±11
3.4	2.2±0.3	1.7±0.4	18±3.8	0.5±0.5	4.2±0.9	3.9±0.9	147±7.1	55±8.7	0.15±0.05	3±1.8	0.93±0.5	12±3.2
2.4	2.9±0.3	1.4±0.4	27±5.5	1.4±1.1	4.7±1.1	4.5±0.2	93±10.8	54±8.0	0.2±0.06	6±1.7	1.5±0.3	8±2.1
1.5	3.8±0.4	2.5±0.2	28±6.2	4.2±1.5	7.5±1.0	6.0±0.2	101±4.9	51±9.5	0.5±0.21	10±4.9	0.6±0.3	11±1.8
1.0	7.3±0.6	6.5±0.8	36±4.5	15.7±1.5	6.2±3.2	5.5±1.5	70±4.2	30±1.5	0.4±0.15	6±3.8	0.8±0.3	9.3±0.8

4.2.2.3.8 Specific Methanogenic Activity (SMA) and F/M Ratio Variations in ABR at Different HRTs. Figure 4.41 shows the SMA values of sludge taken from the middle compartment of ABR during continuous operation of ABR at different HRTs. The SMA is an indicator of methanogenic activity in anaerobic systems. As shown in Figure 4.41, SMA varied between 0.29 and 0.55 g COD-CH_4/ gVSS when HRT decreased from 10.38 days to 1 day. After this HRT SMA remained stable between 0.34 and 0.39 g COD-CH_4/ gVSS at HRTs varied between 1.5 and 5.19 days. SMA was 0.29 g COD-CH_4/ gVSS at a HRT of 1 day. Methanogenic activity decreased at minimum HRT such as 1 day. The reason of this could be explained with high flow rates and OLRs decrease the activity of methanogens. Donlon et al., (1996) reported 0.87 g COD-CH_4/g VSS day in UASB treating 260 mg/l p-NP in at HRT of 0.33 days.

Figure 4.42 shows the variations in F/M ratio in ABR at different HRTs. The F/M ratios increased with decreasing of HRT (R^2=0.66, f=3.1, p=0.15, df=5). F/M ratio increased from 0.005 to 0.06 day^{-1}as HRT decreased from 10.38 days to 1 day The stepwise increase of the F/M ratios was achieved through the decrease of hydraulic retention time (HRT) coupled with the increase of COD and p-NP loading rates. Furthermore the VSS concentrations varied at high and low HRTs. F/M ratio variations depended on COD loading rate (R^2=0.98, f=98.9, p=0.0006, df=5) compered to VSS concentration in reactor (R^2=0.35, f=0.57, p=0.49, df=5). Maximum VSS concentration in ABR was found as 71 g/l at a HRT of 5.13 days. After this HRT, VSS concentrations in ABR decreased 60 g/l at HRTs varied between 3.4 and 2.4. VSS concentration in ABR was 48 g/l at a HRT of 1 day. This can be explained high washouth from the effluent and inhibition effect of p-NP on granulated biomas.

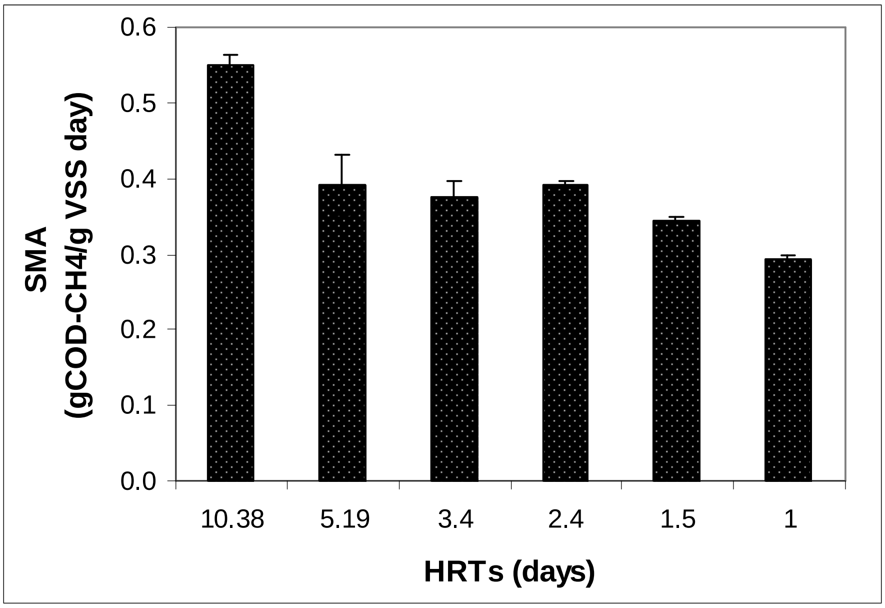


Figure 4.41 SMA values in ABR at different HRTs.

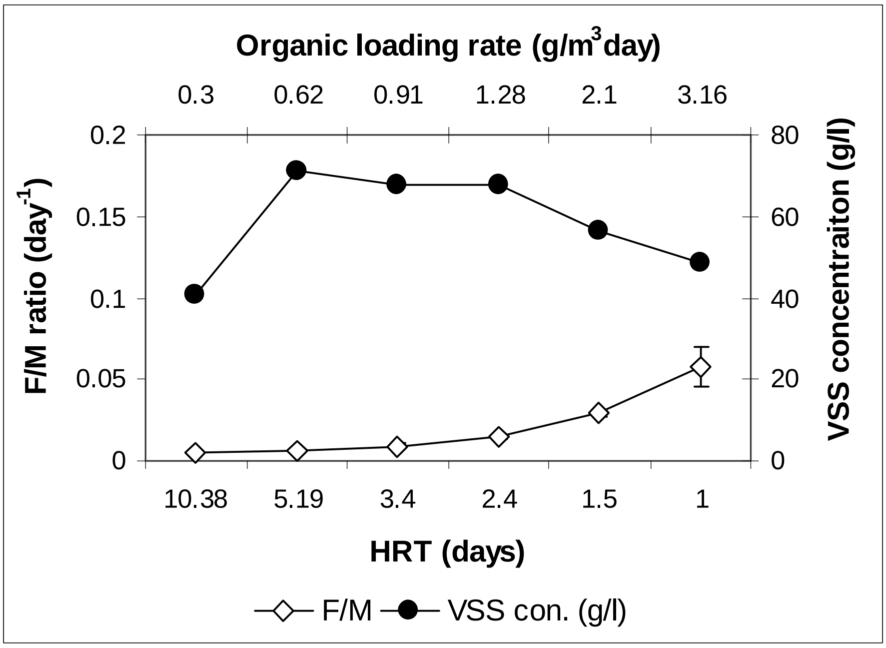


Figure 4.42 F/M ratios and VSS concentrations in reactor in ABR at different HRTs.

4.2.2.3.9 Assessment of Toxicity of Sequential Anaerobic ABR/Aerobic CSTR Reactor System. In this study, toxicity of effluent of ABR and aerobic (CSTR) reactor was determined by bioluminescence test using bacteria *Photobacterium phosphoreum* (LCK 480). Table 4.7 shows the inhibition percentage of samples from the influent synthetic wastewater consisting in a p-NP concentration of 80 mg/l, and anaerobic and aerobic effluent at a HRT of 2.5 days at 5th, 15th, 30th minutes.

LUMIStox test is an acute toxicity test. Therefore the toxicity results of test could be obtained in the short time (maximum 30 min). As shown in Table 4.7, the inhibition percentage (H) of influent was found as 99 % at incubation time of 30 min. These result showed that the wastewater containing 85 mg/l of p-NP was toxic. After anaerobic treatment, the effluent toxicity decreased to 62 % at a incubation time of 30 min (See Table 4.7). This shows that p-NP transformed to less toxic intermediate products under anaerobic conditions. In other words, the intermediate products such as p-AP and phenol were less toxic of its corresponding p-NP. After aerobic treatment, toxicity decreased from 62 % to 28 % in aerobic effluent at incubation time of 30 min. The aerobic reactor effluent was possibly toxic.

Table 4.7 Lumistox toxicity test results in sequential ABR/ CSTR reactor system (HRT=3.4 day).

Inhibition H (%)	**Time (min.)**	**Anaerobic influent**	**Anaerobic effluent**	**Aerobic effluent**
H_5	5	99	60	25
H_{15}	15	99	60.5	27
H_{30}	30	99	62	28

4.2.2.3.10 General Discussion. The pH, TVFA, Bic.Alk. and TVFA/Bic.Alk. ratio obtained from ABR exhibit similar results compered with the date obtained from AMBR. In the initial compartments of the AMBR and ABR reactors were achieved the highest removal efficiencies. COD and p-NP removal efficiencies were up to 90% in the first compartments of AMBR and ABR reactors. The TVFA concentrations in the first compartment of AMBR and ABR reactors were higher than that the other compartments. pH values in the compartments and in the effluent were between optimum values at all p-NP loading rates and all HRTs. TVFA concentration decreased through compartments 2-3. The effluent TVFA concentration was low at all p-NP loading rate and HRTs. TVFA/Bic.Alk. ratio was below 0.4, indicating the stability of the AMBR and ABR reactors.

The COD and p-NP removal efficiencies in ABR reactor were compared to AMBR reactor. It was observed that the ABR reactor exhibits a good performance. Table 4.8 shows the COD and p-NP removal efficiencies, total and methane gas productions, methane percentages, effluent pH, TVFA, Bic.Alk. and TVFA/Bic.Alk. obtained AMBR and ABR reactors treating p-NP at increasing p-NP loading rate and decreasing HRTs. COD and p-NP removal efficiencies in ABR reactor were high (E=79-94%; E=71-99%) and (E=89-96%; E=95-98%) at increasing p-NP loading rates and decreasing HRTs. Total and methane gas productions were higher in ABR than that obtained in AMBR. COD and p-NP removal efficiencis in ABR reactor were found as 93% and 99%, respectively, at p-NP loading rate of 9.63 g/m^{3}day corresponding a p-NP concentration of 100 mg/l. However COD and p-NP removal efficiencies in AMBR reactor were 56% and 90%, respectively, at maximum p-NP loading rate of 9.63 g/m^{3}day corresponding a p-NP concentration of 100 mg/l. AMBR reactor deteriorated above this p-NP concentration. This shows that ABR reactor is more resisted to high p-NP concentrations and at low HRTs compared to AMBR.

Table 4.8 Comparison of AMBR and ABR treating p-NP.

	AMBR reactor		**ABR reactor**	
	p-NP loading rate (g/m^3 day)	**HRT (days)**	**p-NP loading rate (g/m^3 day)**	**HRT (days)**
	0.96- 9.63	**1 - 10.38**	**0.96 - 67.9**	**1 -10.38**
COD removal efficiency (%)	56% -92%	90% -93%	79% -94%	71% -99%
p-NP removal efficiency (%)	90% -93%	86% -95%	89% -96%	95% -98%
The effluent pH	7.7-8.3	7.1-7.4	7.3-7.6	7.0-7.6
The effluent TVFA (mg/l)	55-529	23-177	0	0-560
The effluent Bic.Alk. (mg/l)	3000-3380	3000-3300	2973-3400	2038-3420
The effluent TVFA/Bic.Alk.	0.02-0.21	0.02-0.09	0	0-0.29
Total gas (l /day)	1.45-2.3	2.16-12.25	2.0-2.6	2.2-17.7
Methane gas (l/day)	0.85-1.3	1.02-3.8	1.0-1.3	0.9-4.3
Methane percentage (%)	43-52	30-44	42-54	16-39

4.2.3 The removal of NB in AMBR and Sequential AMBR/CSTR Reactor System

4.2.3.1 Effect of Increasing NB Loading Rates on Performance of AMBR Reactor

4.2.1.1.1 Effects of Increasing NB Loading Rates on COD and NB Removal Efficiencies in Anaerobic AMBR reactor. In this step, the effect of increasing NB loading rate on COD removal efficiencies was investigated. The operation of the AMBR was started with an influent NB concentration of 20 mg/l and at a NB loading rate of 1.93 g/m^{3}day. Then NB concentrations were subsequently increased from 40, 60, 100, 180, 250, 300 to 400 mg/l, at increasing of NB loading rates from 3.85, 5.78, 9.63, 17.34, 24.08, 28.90 to 38.54 g/m^{3}day. The influent COD concentration increased with increased NB concentration since additional NB concentration increase the initial COD concentration. The variations of COD with increasing NB loading rates are shown in Figure 4.43. COD removal efficiency remained approximately between 93 and 94 % until a NB loading rate of 5.78 g/m^{3}day correspond to a NB concentration of 60 mg/l. After this NB loading rates the COD removal efficiency decreased rapidly from 93 % to 84 %. A strong linear correlation between COD removal efficiencies and NB loading rates was observed (R = 0.98; d.f. = 7, F = 269.9, p = 2.43E-06).The effluent COD concentration and removal efficiency were measured as 523 mg/l and 84 %, respectively at maximum NB loading rate of 38.54 g/m^{3}day corresponding to NB concentration of 400 mg/l.

Effect of increasing NB loading rate on the NB removal efficiencies are shown in Fig. 4.44. NB removal efficiency was found as 100 % at all NB loading rates. The effluent NB concentrations were approximately 0 mg/l at all NB loading rates. 100 % of the nitrobenzene was reduced to aniline in the anaerobic reactor at influent NB concentration of 400 mg/l (NB loading rate=38.54 g/m^{3}day) and at a HRT of 10.38 days (See Fig. 4.43). The aforementioned NB concentration is higher than the IC_{50} value obtained for NB concentration (IC_{50}=109 mg/l) causing 50% inhibition on the methane production. This shows that the anaerobic granule microorganisms in AMBR reactor acclimated to high NB concentration. In this study, the optimum NB loading rate and NB concentration were found between 0.96 and 3.85 g/m^{3}day and 20-60 mg/l, respectively, for maximum COD removal efficiency of 93-94% of and

NB removal efficiency of 100%. These results were higher than that performed by Aziz, Ng, & Zhou, (1994). In this study, NB and COD removal efficiencies were 97% and 16%, respectively in acidogen reactor at a HRT of 1 day and an influent NB concentration of 103 mg/l.

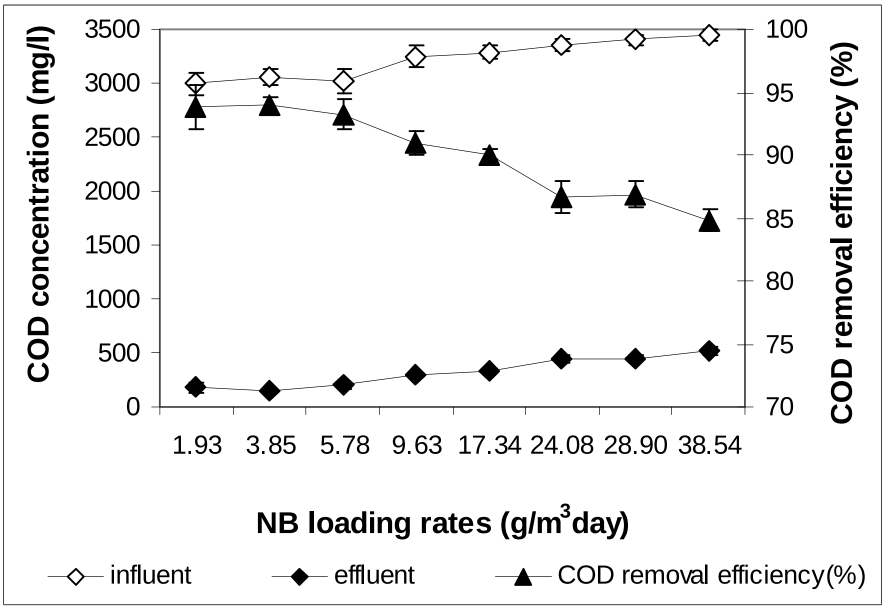


Figure 4.43 Effect of increasing NB loading rates on COD removal efficiencies in AMBR.

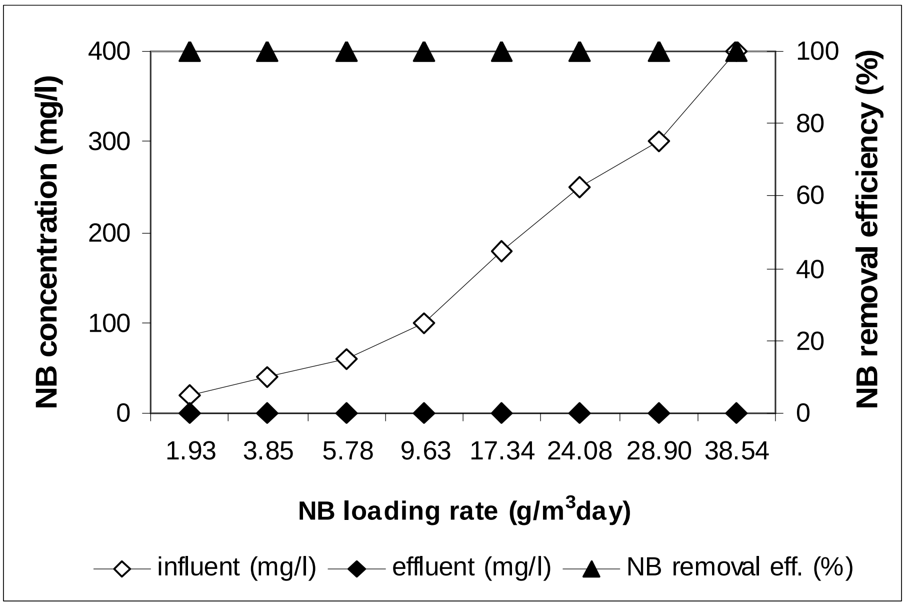


Figure 4.44 Effect of increasing NB loading rates on NB removal efficiencies in AMBR.

4.2.1.1.2 Effects of Increasing NB Loading Rates on the Total and the Methane Gas Production in AMBR Reactor. Figure 4.45 shows the effect of NB loading rate on gas production and methane percentage. The daily total gas, methane gas productions and % methane production were about 2.8 l/day and 1.3 l/day and 44%, respectively until a p-NP loading rate of 5.78 g/m^{3}day. The maximum total gas, methane gas productions and methane percentage were found as 2.8 l/day, 1.3 ml/day and 45 %, respectively, at a p-NP loading rate of 5.78 g/m^{3}day. These datas exhibited similar results with the study performed by Sponza & Kuşçu (2005) in AMBR reactor treating a 40 mg/l of p-NP at a HRT of 10.38 days. In this study, the maximum total gas, methane gas productions and methane percentage were found as approximately 2300 ml/day, 1300 ml/day and 56%, respectively at a p-NP loading rate of 4.81 g/m^{3}day in AMBR reactor (Sponza & Kuşçu, 2005). After a p-NP loading rate of 5.78 g/m^{3}day, the daily total gas, methane gas productions and methane gas percentage began decline. 1.8 l/day, 0.78 l/day and 39% removals were obtained at a maximum NB loading rate of 38.54 g/m^{3}day, respectively, aforementioned parameter. This indicated the inhibition effect of NB on methane *Archeae* at NB loading rates as high as 38.54 g/m^{3}day correspond to NB concentration of 400 mg/l.

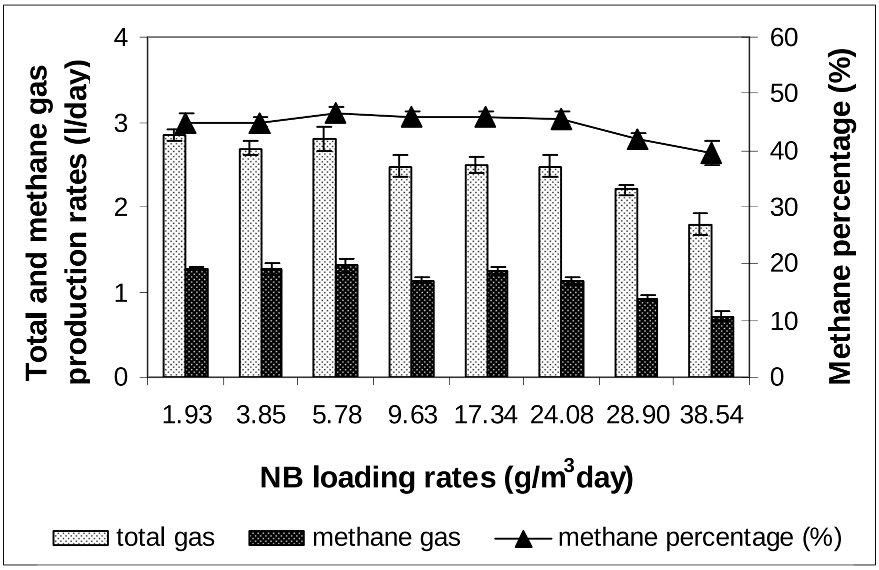


Figure 4.45 Effect of increasing NB loading rate on total gas, methane gas productions and methane percentage in AMBR.

4.2.1.1.3 Variation of COD, NB Removal Efficiencies in Compartments of the AMBR Reactor at Increasing NB Loading Rates. Figure 4.46 (a) and (b) shows the initial remaining COD and NB concentrations in the compartments of AMBR. As shown in Fig. 4.46 (a) and (b), the most of COD and NB were removed in the first compartment. The influent COD concentration varied between 3000 and 3500 mg/l and then decreased to 246 mg/l (92% efficiency) at NB loading rate of 1.93 g/m^{3}day in the first compartment. COD concentration was 1120 mg/l (E=67% removal) in the first compartment at NB loading rate of 38.5 g/m^{3}day. COD removal efficiency decreased with increasing NB loading rates in the first compartment. The COD removal efficiencies were 20-45% in the second compartment, 15-23% in the third compartment at NB loading rates as high as 38.54 g/m^{3}day.

The NB removal efficiencies decreased from 100 % to 99% at high NB loading rates such as 24.08, 28.9 and 38.5 g/m^{3}day in the first compartment (See Fig 4.46(b)). However the NB concentrations in compartment 3 were approximately 0 mg/l at all NB loading rates. NB concentration were 4 mg/l in initial compartment, 0.1 mg/l in second compartment and 0.01 mg/l in third compartment at high NB loading rate of 38.54 g/m^{3}day. These results showed the effect of compartmentalisation on the AMBR reactor performance based on COD and NB removal efficiency.

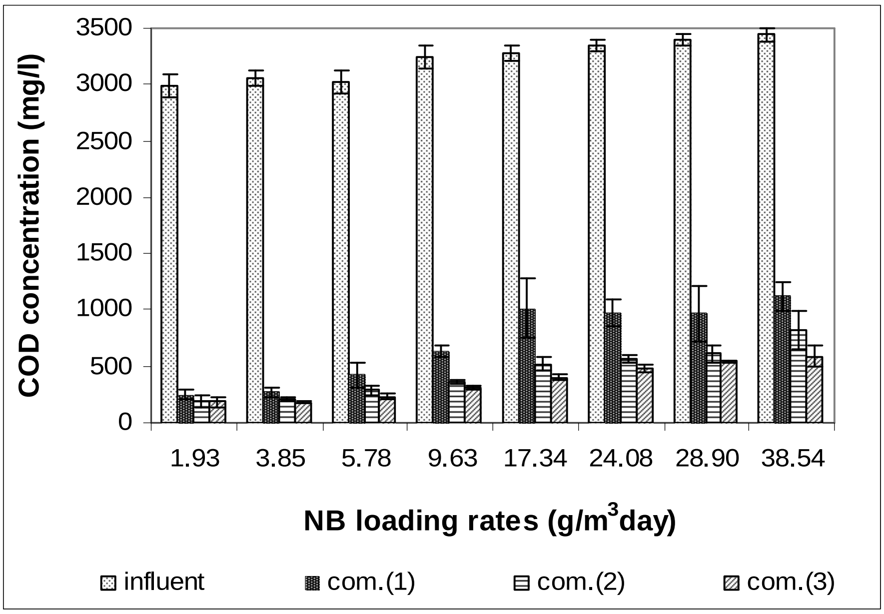


Figure 4.46 The COD concentrations in compartments of AMBR at increasing NB loading rates (a).

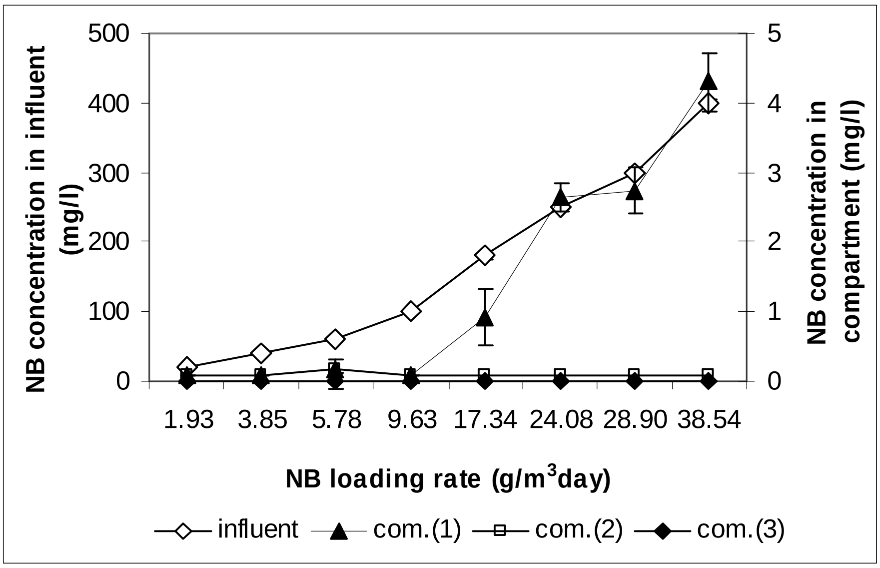


Figure 4.46 The NB concentrations in compartments of AMBR at increasing NB loading rates (b).

4.2.1.1.4 The Variation of pH, TVFA, Bicarbonate Alkalinity (Bic.Alk.) and TVFA/Bic.Alk. ratio in Compartments of the AMBR Reactor at Increasing p-NP Loading Rates. Figure 4.47 shows the pH, TVFA, Bic.Alk. and TVFA/Bic.Alk. ratio variations in compartments of AMBR reactor at increasing NB loading rates. As shown in Figure 4.47 (a), the pH values in the effluent and in the compartments of AMBR varied between 7.0-7.9. These values were between optimum pH values as reported by Speece, (1996). The pH values were lower in initial compartment than that the other compartments since TVFA in the first compartment rising. The pH values varied between 7.2 and 7.4 in initial compartment as the NB loading rates increased from 1.93 to 38.54 g/m^{3}day.

TVFA concentrations were high in the initial compartment at all NB loading rates. As the NB loading rate increased TVFA concentrations increased in the first compartment (See Fig. 4.44 (b)). A strong linear correlation between TVFA concentrations in the first compartment and NB loading rates was observed (R^2 = 0.99; d.f. = 7, F = 218.9, p = 1.59E-05). The TVFA concentration increased from 46 to 160 mg/l in the first compartment as NB loading rate increased from 1.93 to 38.54 g/m^{3}day. The TVFA concentration was zero in second and third compartment of AMBR at NB loading rates of 1.93 and 3.85 g/m^{3}day, respectively. After these NB loading rates, TVFA concentration increased from 10 mg/l to 100 mg/l in second compartment and from 15 mg/l to 60 mg/l in the third compartment as the NB loading rate increased from 3.85 to 38.54 g/m^{3}day. However TVFA concentrations in the effluent of AMBR were zero except for NB loading rates of 28.9 and 38.54 g/m^{3}day. The TVFA concentrations in the effluent were 11 mg/l and 17 mg/l at these loading rates, respectively.

Figure 4.47(c) shows the Bic.Alk. variations in compartments of AMBR reactor at increasing NB loading rates. The Bic.Alk. concentrations were lower in the first compartment than the others compartments due to decline of pH. This indicates the utilization of alkalinity to buffer the TVFA and CO_2 produced from the anaerobic co-metabolism of NB. TVFA/Bic.Alk. ratio gives necessary information to determine the stability of the anaerobic reactor. If the TVFA/Bic.Alk. ratio is lower than 0.4, the reactor is stable. When the TVFA/Bic.Alk. ratio is lower than 0.8, the reactor system is moderately stable or unstable by Behling et al., (1997). As shown in Fig. 4.47(d), this ratio varied between 0.00 and 0.04 in compartments and the effluent of AMBR at increasing NB concentration. These results indicated that AMBR reactor treating NB was stable at increasing NB concentrations.

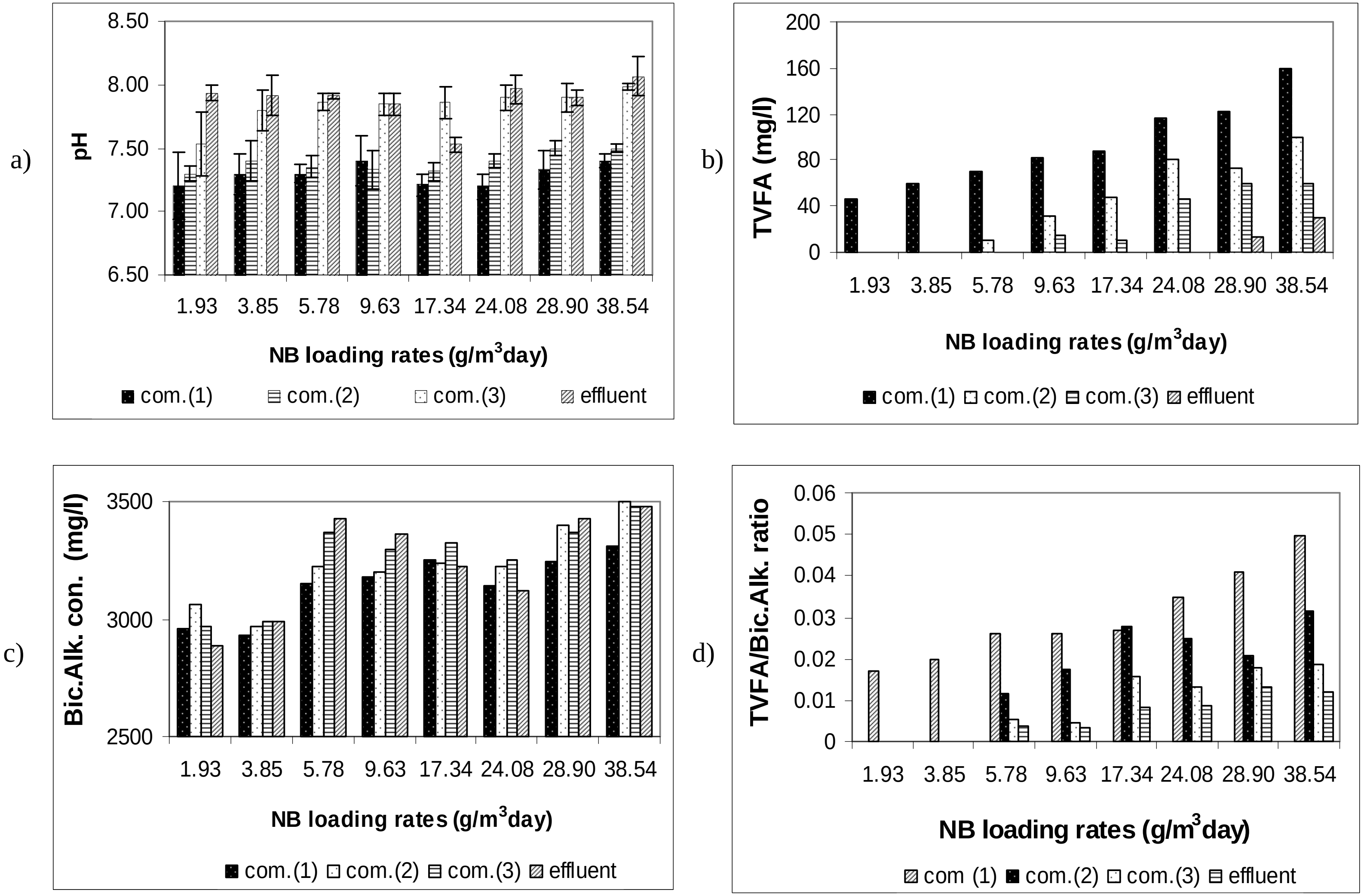


Figure 4.47 The variations of pH (a), TVFA (b), Bic.Alk. (c) and TVFA/Bic.Alk. ratio (d) in AMBR at increasing NB loading rates.

4.2.1.1.5 Determination of Intermediate Products Through NB Degradation Under Anaerobic and Aerobic Condition. Three different mechanisms have been described for the mineralization of NB. The first mechanisms involve its degradation via catechol to 2-hydroxymuconic semialdehyde by *Comamonas* sp. JS765 in an oxidative pathway (He & Spain, 1999; Nishino & Spain, 1993). In the second mechanism, a partial reduction of NB produces hydroxiaminobenzene, which is rearranged to 2-aminophenol, and then to aniline (He & Spain, 1999; Nishino & Spain, 1993; Peres, Naveau, & Agathos, 1998). Afterward, in this mechanism, nitrobenzene converted to hydroxiaminobenzene firstly via nitrosobenzene and then to aniline. The third mechanism is reported under anaerobic conditions. Nitrobenzene is reduced completely to aniline in an anaerobic acidogenic reactor (Aziz, Ng, & Zhou, 1994; Zhao & Ward, 1999). Aziz, Ng & Zhou, 1994 investigated the biodegradability of nitrobenzene using sequential acidogenic-aerobic process. The results of nitrobenzene degradation showed that 1 mol of nitrobenzene reduced to 1 mol of aniline in the acidogenic process. Afterwards, NB transformed completely to aniline under anaerobic conditions. Then aniline was rapidly mineralized in aerobic process. Peres, Naveau, & Agathos, (1998) investigated the biodegradation of NB to aniline in a single reactor. The behavior of the mixed population was indicated that NB was reduced to aniline via hydroxiaminobenzene in first step, and, in the second, oxidative step, aniline was mineralized to catechol with meta cleavage. Our study confirmed the results reported by Aziz, Ng & Zhou, (1994) and Peres, Naveau & Agathos, (1998). In our study, nitrobenzene was reduced to aniline, in the first step, under anaerobic condition, and then in the second step, aniline was mineralized to catechol under aerobic conditions.

Figure 4.48 shows the HPLC chromatogram of aniline standard of 500 mg/l (a), and anaerobic AMBR effluent (b). A peak of aniline standard of 500 mg/l was obtained at a retention time of 3.44 min and at a wave length of 234 nm (See Fig. 4.48 (a)). Similar peak are showed on the chromatograms at the same retention times in the effluent sample of AMBR (See Fig. 4.48 (b)). The presence of aniline peak in effluent of anaerobic AMBR indicated that the nitrobenzene converted to aniline under anaerobic conditions. Peres, Naveau, & Agathos, (1998) found that biodegradation of nitrobenzene to aniline occurred via nitrosobenzene. However no nitrosobenzene peaks were observed in the compartments and effluent of anaerobic AMBR and ABR reactors. Figure 4.48 (c) shows the HPLC chromatogram for nitrosobenzene standard of 120 mg/l. In this study no nitrosobenzene peak was observed in HPLC analysis. This shows that NB is reduced completely to aniline under anaerobic conditions as reported by Aziz, Ng, & Zhou, 1994. Aniline was biodegraded in aerobic stage. No peak of aniline was observed in the effluent of aerobic CSTR reactor (See Fig. 4.49 (a)). Catechol was observed in the oxidative step as reported by Peres, Naveau, & Agathos (1998). Fig. 4.49 (b) shows the peak of catechol standard of 200 mg/l at a retention time of 2.95 min and at a wave length of 234 nm. This showed that aniline was biodegraded to catechol in aerobic stage. Peres, Naveau, & Agathos (1998) reported that catechol mineralized to 2-Hydroxymuconic semialdehyde via catechol-2,3-dioxygenase and then joined to TCA (tricarboxylic acid) cycle. In our study, the metabolic pathway of nitrobenzene through sequential anaerobic/aerobic stage was illustrated in Figure 4.50.

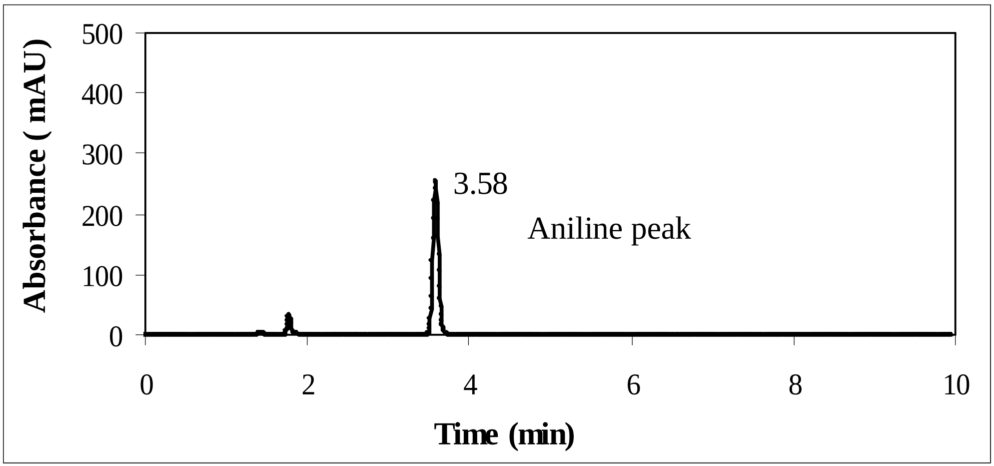


Figure 4.48 HPLC chromatogram of aniline standard (a) (aniline concentration =500 mg/l, retention time=3.44 min, wave length= 234 nm) (mAU=mili amper unit).

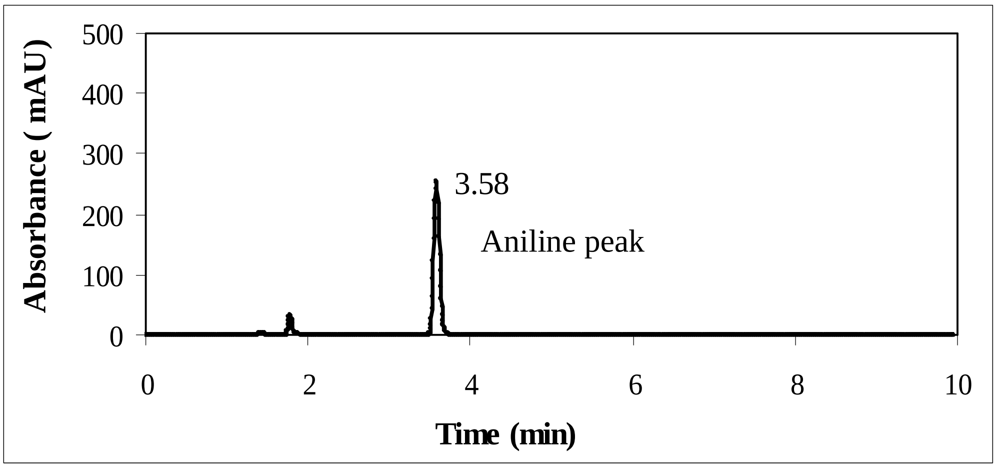


Figure 4.48 HPLC chromatogram of anaerobic AMBR effluent (HRT=10.38 days; influent NB concentration = 400 mg/l, retention time=3.44 min, wave length= 234 nm) (mAU=mili amper unit) (b).

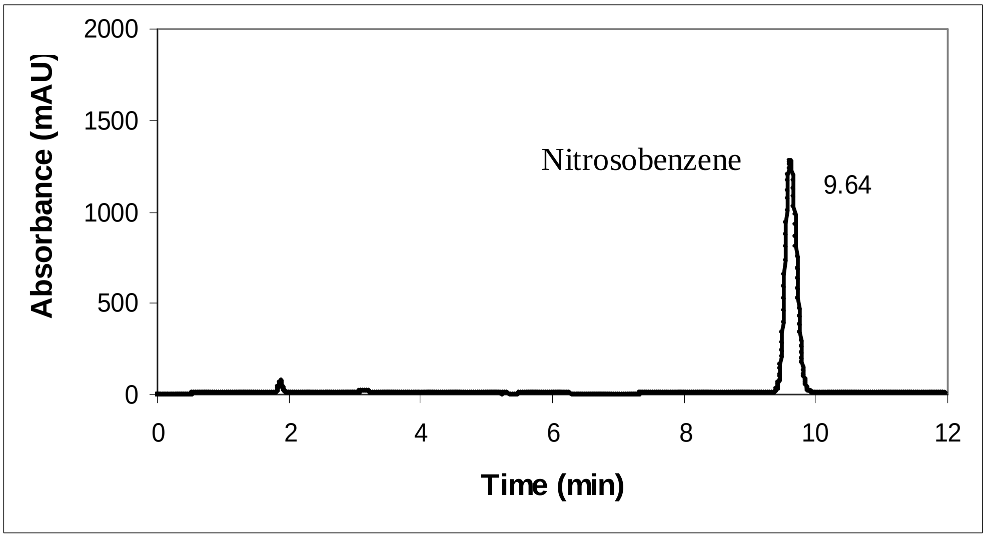


Figure 4.48 HPLC chromatogram of nitrosobenzene standard (nitrosobenzene concentration=120 mg/l, retention time=9.64 min, wave length= 234 nm, mAU=mili amper unit) (c).

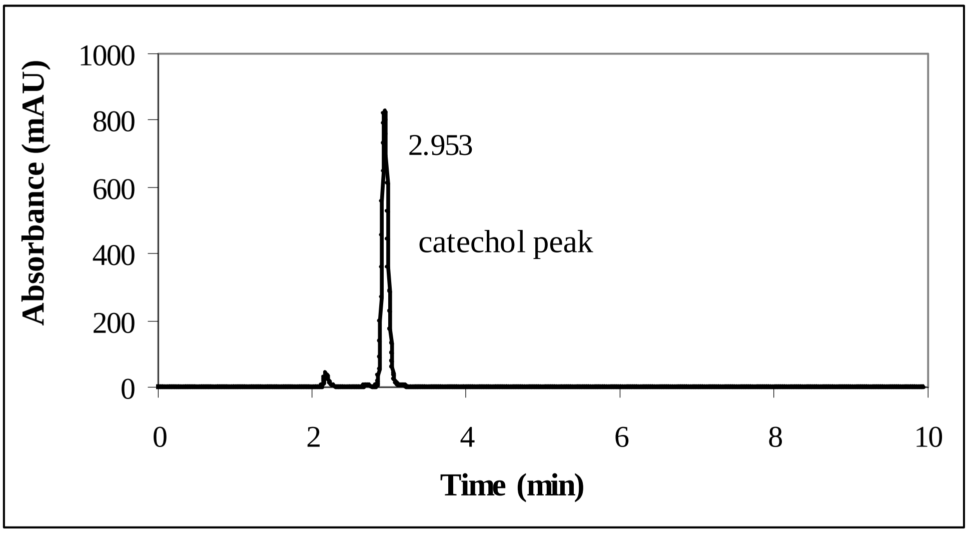


Figure 4.49 HPLC chromatogram of aerobic CSTR reactor effluent (HRT= 6.9 days, retention time=2.95 min, wave length= 234 nm; mAU=mili amper unit) (a).

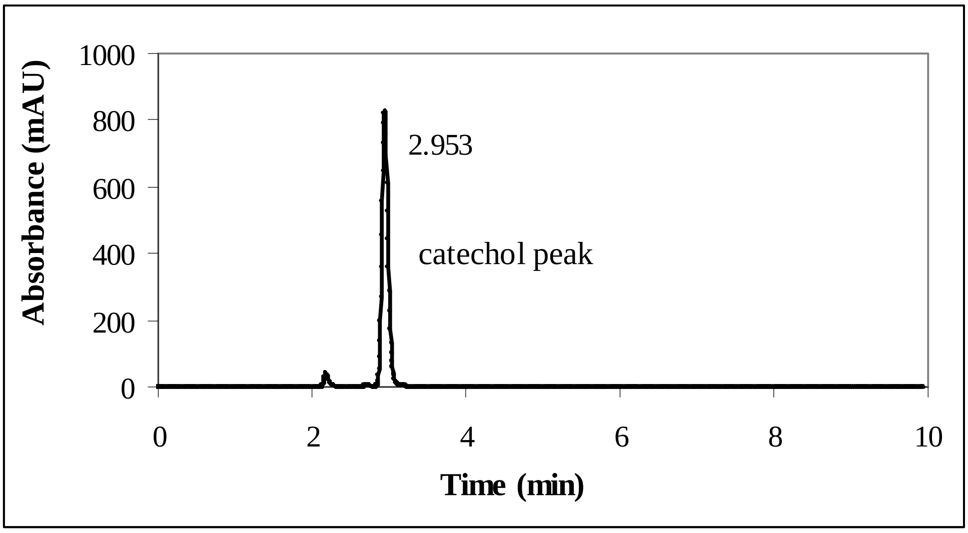


Figure 4.49 HPLC chromatogram of catechol standard (catechol concentration =120 mg/l, retention time=2.95 min, wave length= 234 nm ; mAU=mili amper unit) (b).

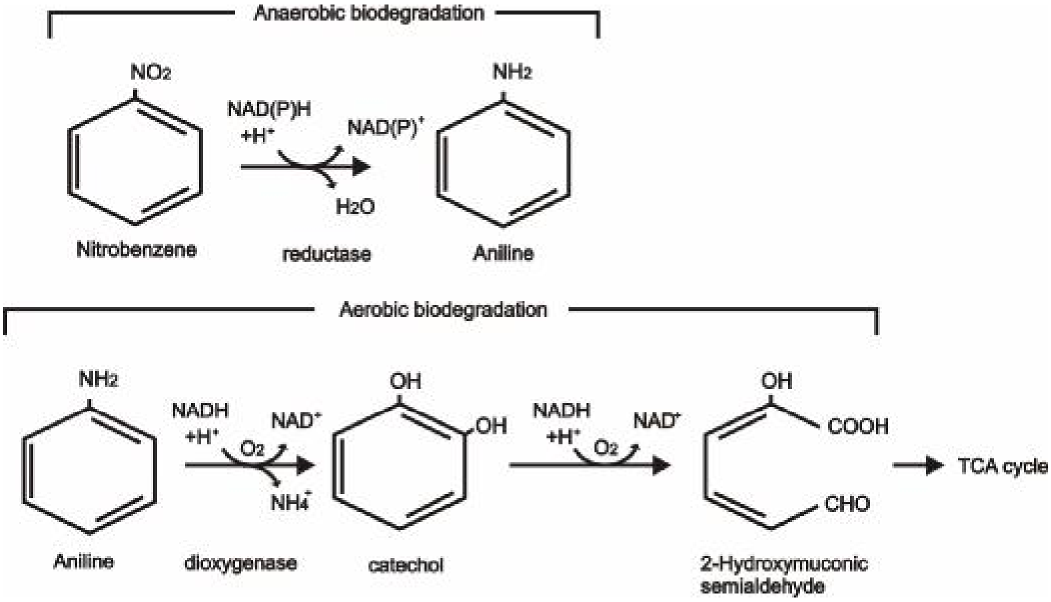


Figure 4.50 Degradation pathway of nitrobenzene in anaerobic/aerobic stage.

4.2.1.1.6 Aniline Production in AMBR Reactor. Figure 4.51 shows the aniline concentrations in the effluent of AMBR at increasing NB loading rates. As shown in this Figure, the effluent aniline concentration increased whenever influent NB concentration increased. A strong linear correlation between influent NB concentration and the effluent aniline concentration was observed ($R^2 = 0.97$; d.f. = 7, F = 202.95, p = 0.88). Aniline concentration in the effluent increased from 7 mg/l to 155 mg/l when NB concentration in the influent increased from 20 to 400 mg/l. This shows that NB was biodegraded to aniline under anaerobic conditions. Stoichiometrically 1 mol NB produces 1 mol of aniline. Therefore 20 mg/l NB produces 19.43 mg/l of aniline. As shown in Figure 4.51, the aniline concentration in the effluent of anaerobic was lower than the stoichiometrically aniline values. This shows that aniline was degraded under anaerobic conditions. Peres, Naveau, & Agathos (1998) indicated that not only nitrobenzene transformation occurred in anaerobic condition but also aniline mineralization can occur under anaerobic condition. This study confirmed our study.

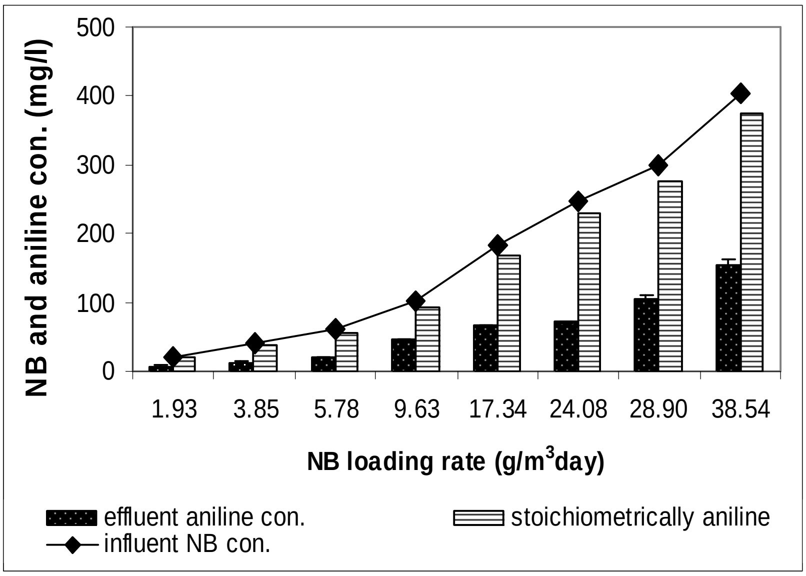


Figure 4.51 Variation of NB in influent samples and aniline concentration in the effluent samples, and stochiometric aniline concentrations at increasing NB loading rates.

4.2.1.1.7 Performance of Aerobic Reactor and Treatment Efficiencies of Anaerobic/Aerobic Sequential Reactor System. Figure 4.52 shows the aniline removal efficiencies in aerobic reactor. NB removal was not observed in aerobic reactor since NB was removed completely in anaerobic reactor (See Fig 4.46 (b)). The remaining COD and aniline were removed in aerobic reactor. Aniline removal efficiencies were 100% until a NB loading rate of 17.34 g/m^{3}day in aerobic reactor. After this loading rate, aniline removal efficiency decreased to 90% at NB loading rate of 38.54 g/m^{3}day. NB loading rate of 38.54 g/m^{3}day was the optimum value for % 100 aniline removal in aerobic CSTR reactor. Figure 4.53 shows the COD removal efficiency in aerobic reactor and in sequential anaerobic/aerobic reactor system. COD removal efficiencies were approximately 50% at all NB loading rates in aerobic CSTR reactor. COD removal efficiencies were found between 93 and 97 % with increasing NB loading rates in sequential anaerobic AMBR/aerobic CSTR reactor system. COD concentration in the effluent of aerobic reactor was 240 mg/l at maximum loading rate of 38.54 g/m^{3}day.

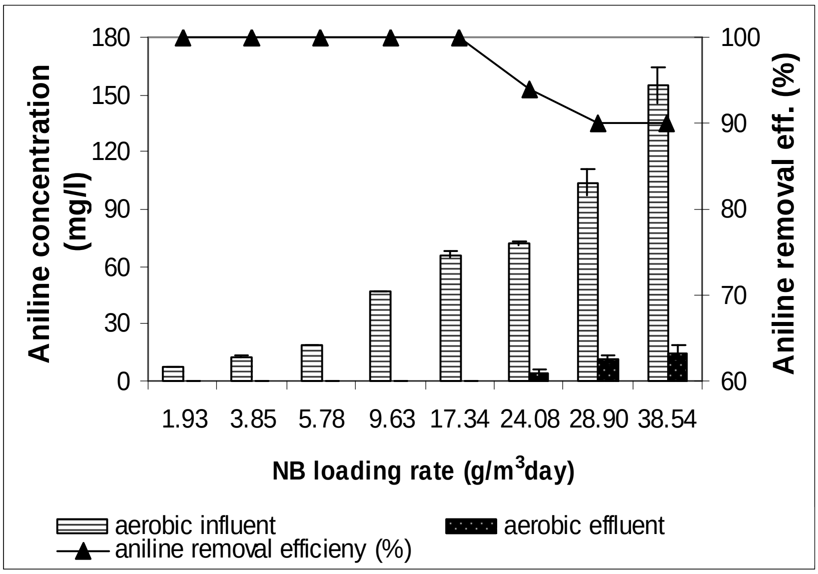


Figure 4.52 Aniline removal in aerobic CSTR reactor at increasing NB loading rates (HRT=6.9 days).

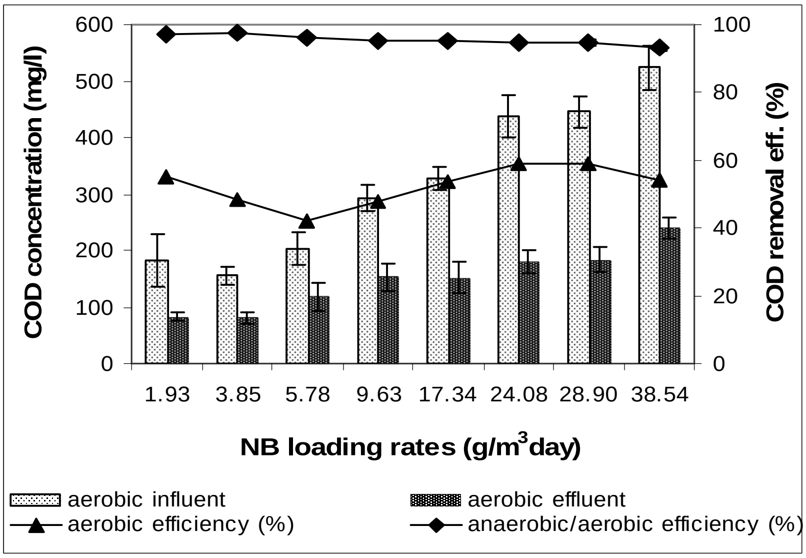


Figure 4.53 COD removal in aerobic reactor (HRT= 6.9 days) and sequential anaerobic (AMBR)/aerobic (CSTR) reactor (HRT=17.28 days) at increasing NB loading rates.

4.2.3.2 Effect of Hydraulic Retention Time (HRT) on the Performance of AMBR Reactor

4.2.3.2.1 Effects of HRT on COD and Nitrobenzene Removal Efficiencies in Anaerobic AMBR Reactor. The effect of HRT on the COD and NB removal efficiencies in AMBR are shown in Figure 4.54. The influent COD and NB concentrations were kept constant at 3000 mg/l and 60 mg/l, respectively, through continuous operation. 92% COD and 100 % NB removal efficiencies were obtained at a HRT of 10.38 days (See Fig. 4.54 a, b). NB removal efficiency was 100 % through all HRTs (See Fig. 4.54 (b)). The optimum COD removal efficiency was found as 92% at a HRT of 5.19 days. The COD removal efficiency showed a falling tendency after a HRT of 3.55 days. When HRT was decreased from 3.55 to 2 days, the COD removal efficiency decreased from 90% to 85% (See Fig. 4.54 (a)). The COD removal efficiency was found as 79% at minimum HRT of 1 day. The effluent COD concentration was 665 mg/l at a HRT of 1 day. The COD removal efficiency decreased from 92% to 82% as HRT decreased from 10.38 days to 1 day. However no variation was observed in NB removal efficiency through all HRTs. NB was completely removed even at short HRT such as 1 day in AMBR reactor. NB concentrations were approximately zero in the effluent of AMBR at all HRTs. The reason of low COD removal efficiency (E=79%) compared to NB was the accumulation of aniline in AMBR. In other words, the NB biodegraded under reductive anaerobic conditions at very short HRTs but the breakdown product, aniline, was not ultimately metabolized and measured as residual COD. In this study, the maximum COD and NB removal efficiencies were found as 92% and 100% at HRTs varying between 5.19-10.38 days. Figure 4.55 shows the HPLC chromatogram of aniline from the samples taken in the effluent of AMBR at a HRT of 1 day. The peak for NB standard was obtained at a retention time of 7.78 min. (See Figure 4.56). NB peak was not observed in chromatograms of the samples taken from effluent of AMBR at different HRTs. This shown that NB was degraded completely to aniline at HRTs varied between 1 day and 10.38 days. In the effluent samples of AMBR reactor aniline peak was obtained at retention time of 3.44 min. The small peak obtained at retention time of 1.9 min could be defined as methanol peak (Dekker, 1991).

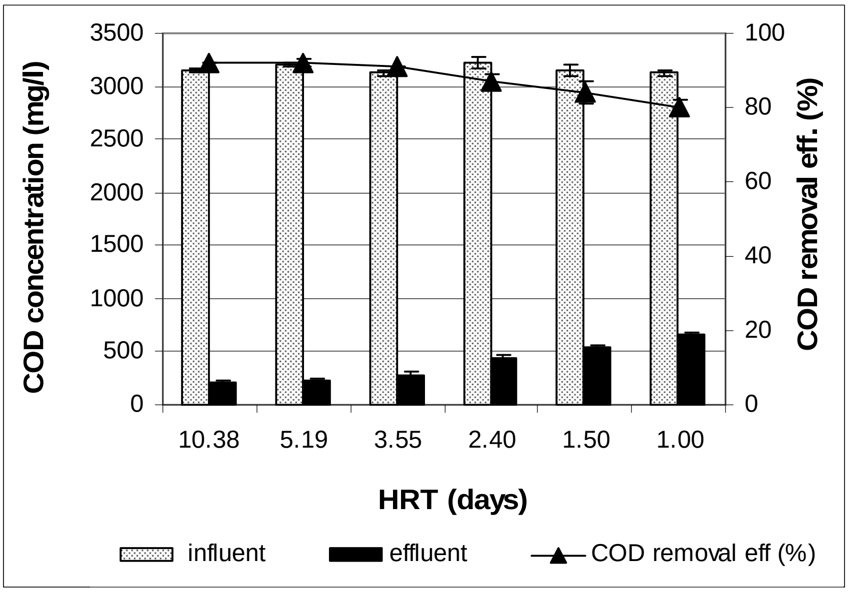


Figure 4.54 The effect of HRT on the COD removal efficiencies in AMBR (a).

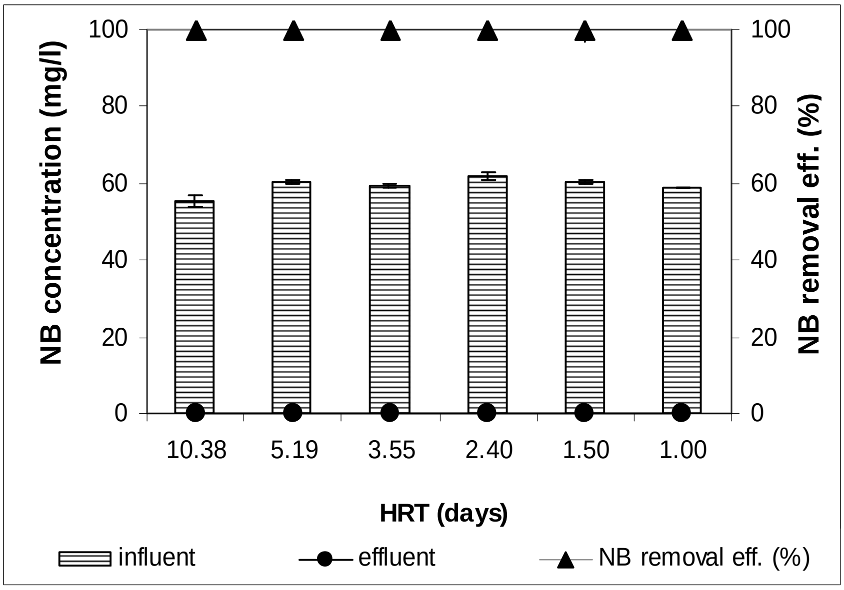


Figure 4.54 The effect of HRT on the NB removal efficiencies in AMBR (b).

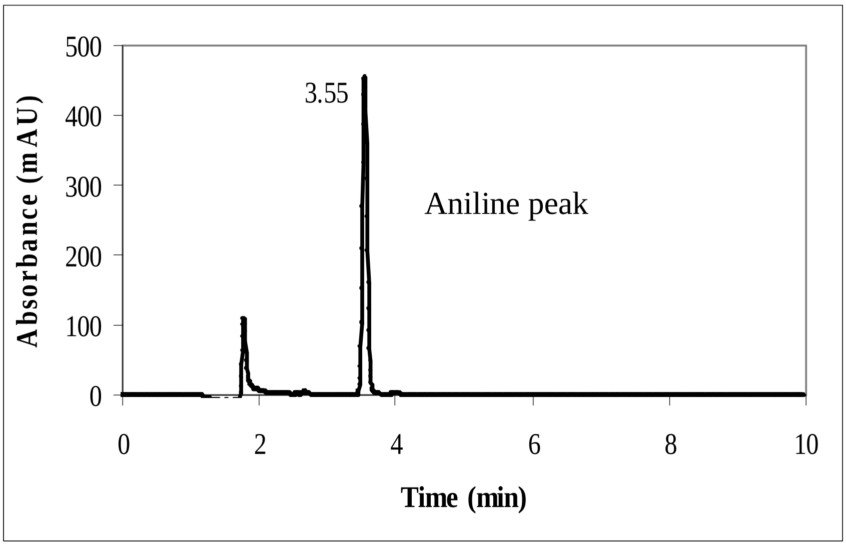


Figure 4.55 The HPLC chromatograms of effluent of AMBR (HRT= 1 day, retention time=3.55 min, wave length= 234 nm) (mAU=mili amper unit).

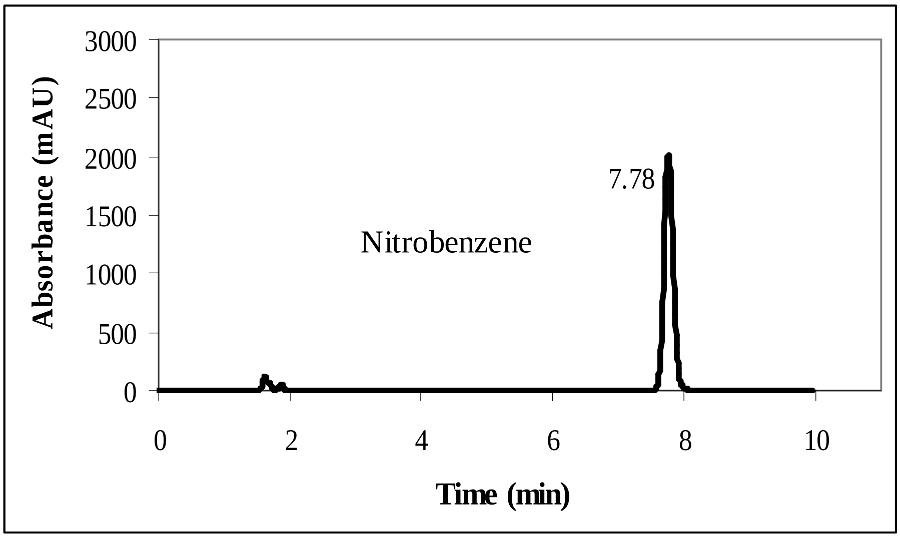


Figure 4.56 The HPLC chromatograms of NB standard (NB concentration =500 mg/l, retention time=7.78 min, wave length= 202nm) (mAU=mili amper unit).

4.2.3.2.2 Effect of HRT on the Total and the Methane gas productions in AMBR Reactor. Figure 4.57 shows the variations of the total, methane gas productions and methane gas percentage in AMBR at all HRTs. From Fig. 4.57, it can be seen that daily total gas and methane gas productions increased from 2.76 to 11.7 l/day and from 1.3 to 3.3 l/day as the HRT was decreased from 10.38 days to 1 day. However, methane gas percentage decreased from 47% to 31% with decreasing HRT. A strong linear correlation between HRT and total gas production, methane gas production, methane percentage were observed. (R^2 = 0.88; d.f. = 5, F = 13.29, p = 0.02; R^2 = 0.96; d.f. = 5, F = 54.58, p = 0.002; and R^2 = 0.78, d.f. = 5, F = 6.43, p = 0.064). Maximum total and methane gas production and methane percentage were observed as 5.6 l/day and 2.6 l/day and 46%, respectively, at HRT of 3.5 days. After this HRT, methane percentage began rapidly decline from 46% to 31%. This can be explained by the partial dominancy of acidogenesis compared to methanogenesis at low HRT in AMBR.

As shown in the Figure 4.58, methane yield (Y) decreased as HRT decreased. Methane yield (Y) decreased from 0.159 to 0.039 m^3CH_4/kg COD removed when the HRT was decreased from 10.38 to 1 day**.** A strong linear correlation between HRT and methane yield (Y) was observed (R^2 = 0.94 d.f. = 5, F = 29.79, p = 0.005). Kuscu & Sponza, (2006) reported that the methane yields decreased from 0.26 to 0.11m^3 CH_4/kg COD, when the HRTs were increased from 10.38 days to 1 day in AMBR reactor treating p-NP. This results exhibits similar results with in AMBR reactor treating 60 mg/l of NB.

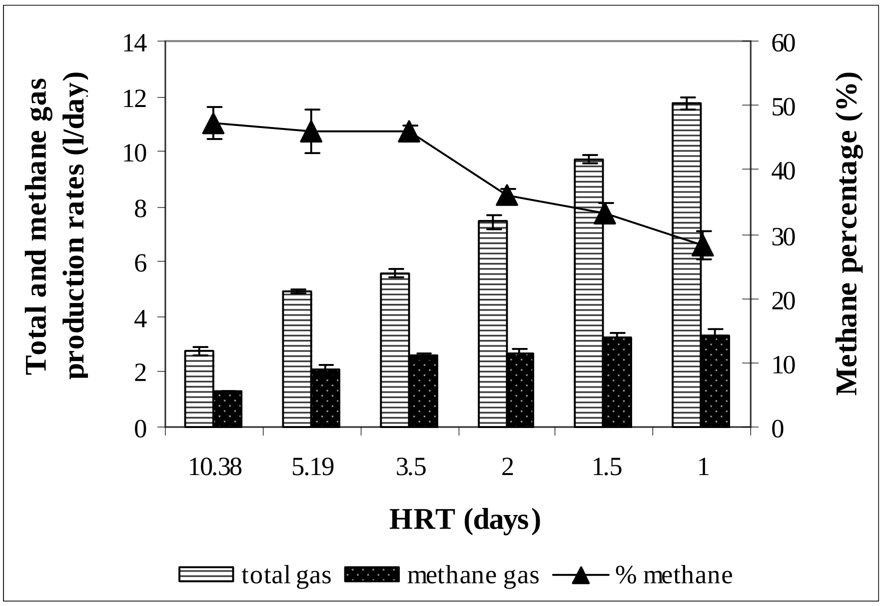


Figure 4.57 The effect of HRTs on total gas, methane gas productions and methane percentage in AMBR.

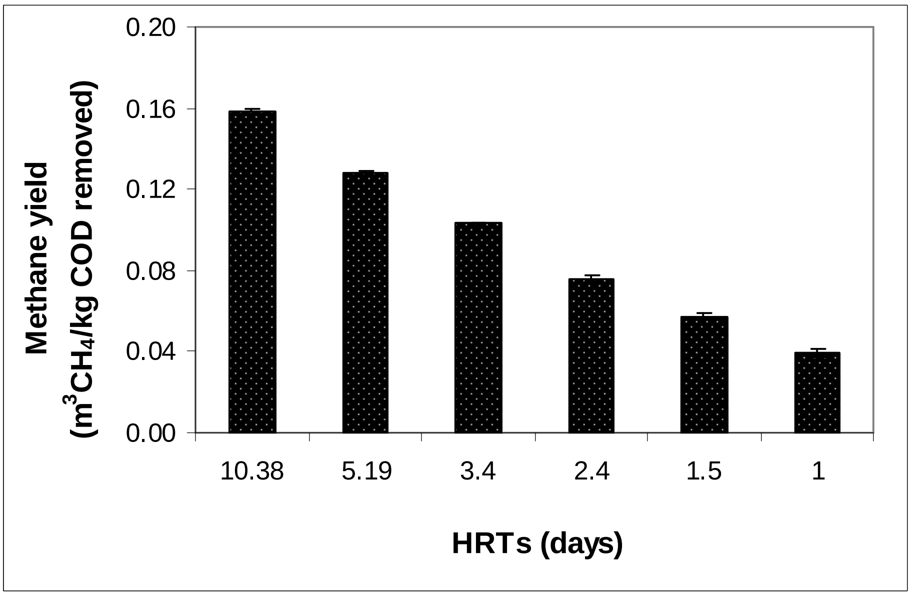


Figure 4.58 The effect of HRTs on methane yield (Y) in AMBR.

4.2.3.2.3 Effect of Compartments in AMBR on COD and NB removal Efficiencies. Table 4.9 shows the COD variations and COD removal efficiencies in compartment of AMBR at different HRTs. As shown in this table, the most of the influent COD was removed in compartment 1. The COD removal efficiency in this compartment varied between 63% and 81% with decreasing HRTs. COD removal efficiencies were 81%, 42% and 23% in compartments 1, 2 and 3 respectively, at a HRT of 1 day. The COD removal efficiency in compartment 2 varied between 18–45% at HRTs varied between 10.38 days and 1 day. A few amount of COD removal (10–20%) was measured in compartment 3. Table 4.10 shows the NB variations in compartment of AMBR at different HRTs. NB concentrations were approximately zero until a HRT of 2 days corresponding to 100 % removal efficiency in the first compartment of AMBR. NB concentrations were 1.72 mg/l and 2.1 mg/l at HRTs of 1.5 days and 1 day, respectively in the first compartment. This could be explained by high NB loading rate at lower HRTs. NB concentrations were found as zero in compartment 2 and 3 and effluent of AMBR at all HRTs. This showed the positive effect of compartmentalisation on COD and NB removal efficiencies in AMBR.

Table 4.9 Variations of COD concentrations and removal efficiencies in compartments of AMBR.

HRT (day)	**COD concentration (mg/l) and COD removal efficiency (%) in compartments (mean±SD)**					
	compartment 1		**compartment 2**		**compartment 3**	
	concentration (mg/l)	efficiency (%)	concentration (mg/l)	efficiency (%)	concentration (mg/l)	efficiency (%)
10.38	610±42	81±1	355±49	42±3	280±32	23±1
5.19	685±49	79±1	388±18	43±5	247±24	11±2
3.5	735±78	76±2	405±21	45±2	333±33	14±1
2	795±49	75±1	515±64	35±3	494±51	10±4
1.5	875±64	72±2	720±57	18±0	600±43	10±1
1	1150±71	63±2	910±71	21±1	755±35	12±1

Table 4.10 Variations of NB concentration in compartments of AMBR.

HRT (day)	**NB concentration (mg/l) (mean±SD)**				
	influent (mg/l)	compartment 1 (mg/l)	compartment 2 (mg/l)	compartment 3 (mg/l)	effluent (mg/l)
10.38	59±2	0.01	0.0	0.0	0.0
5.19	61±1	0.01	0.0	0.0	0.0
3.5	60±1	0.01	0.0	0.0	0.0
2	62±1	0.01	0.0	0.0	0.0
1.5	61±1	1.72±0.5	0.0	0.0	0.0
1	59±1	2.1±0.8	0.0	0.0	0.0

4.2.3.2.4 Aniline Variation in AMBR at Different HRTs. Aniline concentrations were around 45 mg/l in the effluent of AMBR at all HRTs (See Table 4.11). Aniline concentrations varied between 46 mg/l and 49 mg/l in compartment 1, between 42 mg/l and 48 mg/l in compartment 2, between 40 mg/l and 48 mg/l in compartment 3 and between 40 mg/l and 48 mg/l in the effluent of AMBR at HRTs varied from 10.38 days and 1 day. Stoichiometrically 60 mg/l of NB produce 56 mg/l aniline. As seen in Table 4.11, 49 ±1.5 mg/l of aniline produced in first compartment. This shows that 60 mg/l of NB biodegraded to aniline in anaerobic reactor. The sligh decreases of aniline in compartment 2,3 and effluent samples showed that aniline was slightly consumed by the NB degrading methanogenic *Archaea* or a small part of aniline was measured as COD.

Table 4.11 Variations of aniline concentration in effluent and compartments of AMBR.

HRT (days)	**Aniline concentration (mg/l)**				
	influent	**compartment 1**	**compartment 2**	**compartment 3**	**effluent**
10.38	0	47±2	45.9±2.7	45.8±2.5	44.5±2.1
5.19	0	46±1	42.1±0.1	40.1±0.1	40.0±0.0
3.5	0	49±1.5	47.7±1.0	48.1±0.1	48.0±1.4
2	0	48±0	48.1±0.1	47.5±0.6	46.0±0.0
1.5	0	48±1	46.1±0.1	46.1±0.1	45.5±0.7
1	0	47±1	46.9±0.1	46.6±0.6	46.5±0.7

4.2.3.2.5 Effects of HRTs on pH, TVFA and Bicarbonate Alkalinity (Bic.Alk.) Variations in Every Compartment of the AMBR Reactor. pH, alkalinity and volatile fatty acids are integral expressions of the acid-base conditions in anaerobic microbial treatment process, as well as an intrinsic index of the balance between two of the most important microbial groups, the obligate methanogens and the aceticlastic methanogens (Azbar & Speece, 2001). Methanogens prefer nearly neutral pH conditions with a generally accepted optimum range of approximately 6.5 to 8.2. The conditions above or below of this range decrease the rate of methane production. The variations of pH, TVFA, Bic.Alk. and TVFA/Bic.Alk. in the compartment and effluent of ABMR reactor for different HRTs was showed in Figure 4.59 (a, b, c, d). As shown in Figure 4.59 (a), the pH values in the effluent and in the compartments of AMBR varied between 6.8 and 7.9, which are at the optimum pH values for anaerobic treatment. The pH values in the first compartment were lower than the other two compartments. Low pH conditions may be caused by two sources of acidity; H_2CO_3 and volatile fatty acid (VFA), which are generated during the anaerobic microbial reactions (Speece, 1996). Figure 4.59 (b) shows that the highest TVFA concentration was found in the first compartment. High TVFA concentration indicates the acidogenic activity. TVFA concentration in the compartment 1 varied between 167 mg/l and 784 mg/l as the HRT decreased from 10.38 days to 1 day. TVFA concentration decreased in compartments 2 and 3 at all HRTs. TVFA concentrations in effluent of AMBR measured as zero until a HRT of 3.5 days. The TVFA concentrations in effluent of AMBR increased from 150 mg/l to 350 mg/l, when the HRTs were decreased from 2 days to 1 day. This shows that the produced TVFA could not be converted to methane and accumulation of acids occurred at low HRTs. Low VFA concentration indicates a stable reactor performance. In anaerobic compartmentalized reactor, the first compartment is referred as ‘‘acid fermentation’’ and involves the production of TVFAs while the second phase is referred as ‘‘methanenisation. Since the TVFAs are converted to methane and carbon dioxide (Barber & Stuckey, 1999; Azbar & Speece, 2001).

Alkalinity is one of the most central concepts because it controls the pH and thus is a measure the capacity of an aquatic system to buffer the pH in the presence of acids (Speece, 1996). Therefore a sufficient bicarbonate alkalinity must be present to neutralize wastewater. If the acid concentrations (H_2CO_3 and TVFA) exceed the available alkalinity, the reactor will sour, severely inhibiting the methanogens (Speece, 1996). It was found that an alkalinity/COD ratio of 0.5 in the effluent decreased the pH to 6.6 which is considered as the lower limit recommended for the anaerobic digestion processes (Souza, Fuzaro, & Polegato, 1992; Moosbruger, Wentzel, Ekama, & Marais, 1993). In these wastewaters the alkalinity requirement is 1.2–1.6 g alkalinity as $CaCO_3$/g COD $_{\text{influent}}$ which is sufficient to maintain the pH above 6.6 (Speece, 1996). 5000 mg/l of $NaHCO_3$ alkalinity (for 3000 mg/l glucose-COD) was added in feed wastewater to provide an optimum pH in AMBR. The Bic.Alk./COD $_{\text{influent}}$ ratios varied between 0.94 and 1 in AMBR at HRTs varying between 10.38 and 1 day. Figure 4.59 (c) shows the Bic.Alk. concentrations in compartments and in the effluent of AMBR. The Bic.Alk. was measured between 3000-3200 mg/l in the influent of AMBR. The Bic.Alk. concentrations in effluent were found as 2300 mg/l at a HRT of 1.5 day and 1890 mg/l and at a HRTs of 1 day. The reason for low Bic.Alk. concentrations at low HRTs compared to high HRTs could be explained by the accumulation of TVFA (See Fig.4.59 (b)). Figure 4.59 (d) shows the TVFA/Bic.Alk. ratios in compartments and in the effluent of AMBR. TVFA/Bic.Alk. ratios were found below 0.4 in all compartments and in the effluent of AMBR, which indicates the stability of AMBR (Behling et al., 1997).

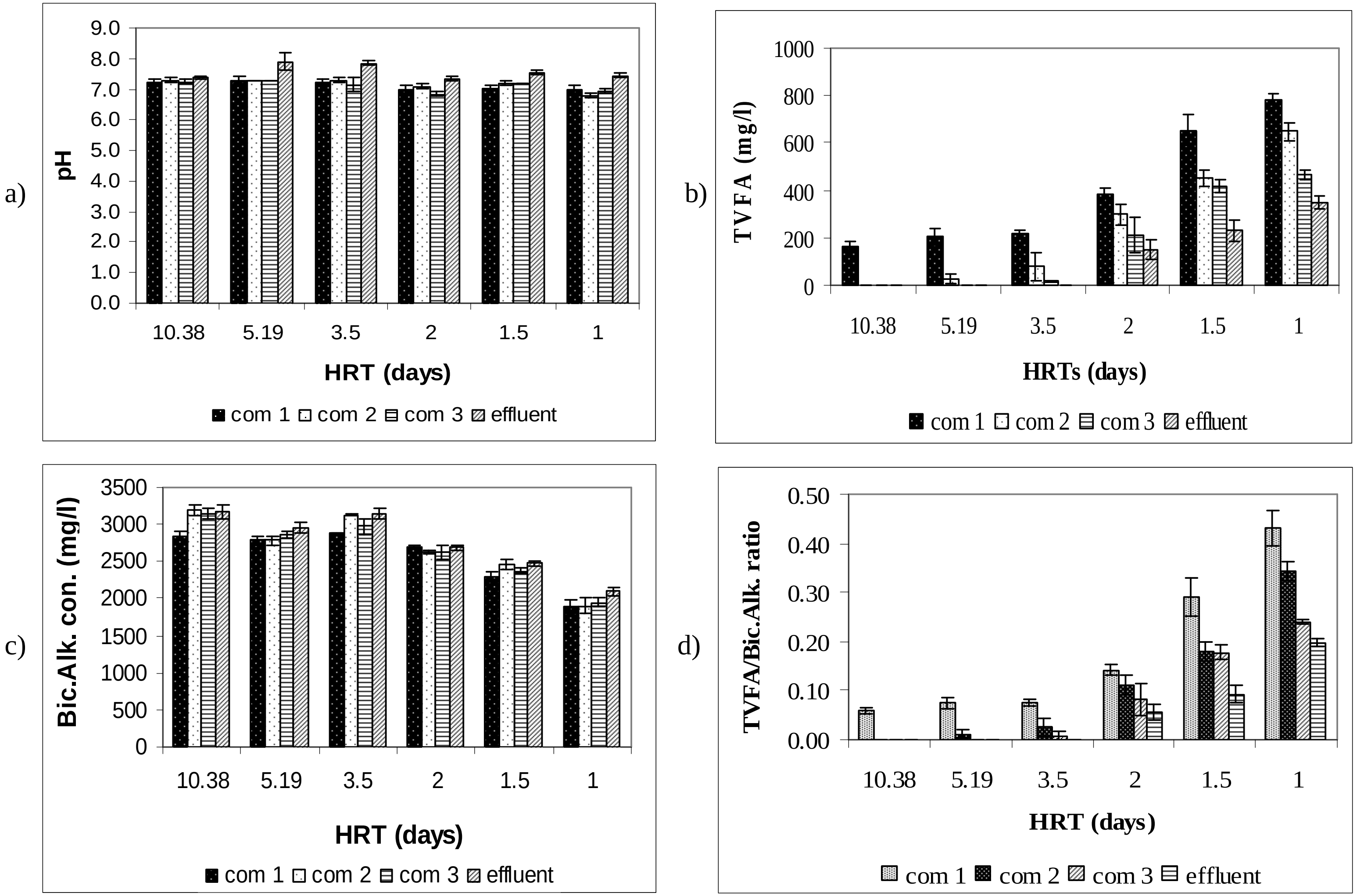


Figure 4.59 The variations of pH (a), TVFA (b), Bic.Alk. (c) and TVFA/Bic.Alk. ratio (d) in AMBR reactor for decreasing HRT.

4.2.3.2.6 Performance of Aerobic CSTR Reactor and Sequential Anaerobic AMBR/Aerobic CSTR Reactor System. Table 4.12 shows the performance of the aerobic process. 62-72% of residual COD remaining from the anaerobic reactor was removed in aerobic reactor. The effluent COD concentration increased from 59 mg/l to 170 mg/l in the aerobic effluent as the HRT decreased from 6.92 days to 0.67 days in aerobic reactor. No NB removal was observed in aerobic reactor since NB removed completely in anaerobic reactor. Aniline removal efficiency was 100% at all HRTs except for 1 day and 0.67 days of HRTs in aerobic reactor. These results were significantly higher than the study performed by Aziz, Ng, & Zhou, (1994) which they found 60% aniline removal efficiency in aerobic step.

Figure 4.60 shows the influent and effluent COD concentrations and COD removal efficiencies in aerobic reactor and in sequential anaerobic/aerobic reactor system at decreasing HRTs. COD removal efficiencies in the aerobic reactor varied between 62 % and 72 % at all HRTs. COD removal efficiencies was approximately 69% at a HRT of 1.33 days. After these HRTs, COD removal efficiencies decreased to 62%. The effluent COD concentration was 225 mg/l at a HRT of 0.67 day in aerobic reactor. COD removal efficiencies in the total system varied between 93% and 98% at all HRTs. The COD (See Fig.4.60) and NB (See Fig.4.54) removal efficiencies (E=98% and 100%, repectively) were higher than results reported by Majumder & Gupta, (2003). They observed 95% COD and 98% NB removal efficiencies at a HRT of 29.5 h in hybrid reactor consisting from trickling filter and an activated sludge units.

Table 4.12 Performance of the aerobic CSTR reactor (mean± SD).

HRT in aerobic reactor (day)	**COD (mg/l)**			**Nitrobenzene (mg/l)**			**Aniline (mg/l)**		
	Inf.	**Eff.**	**Removal (%)**	**Inf.**	**Eff.**	**Removal (%)**	**Inf.**	**Eff.**	**Removal (%)**
6.92	215±21	59±5	72±0.8	0.0	0.0	0	44.5±2.1	0	100
3.46	220±28	84±7	62±0.8	0.0	0.0	0	40.0±0.5	0	100
2.37	288±25	108±35	62±0.4	0.0	0.0	0	49.0±1.4	0	100
1.33	445±22	138±25	69±0.5	0.0	0.0	0	46.0±0.6	0	100
1.00	540±31	170±14	68±0.5	0.0	0.0	0	45.5±0.7	5	89
0.67	665±35	225±21	66±0.5	0.0	0.0	0	46.5±0.7	10	78

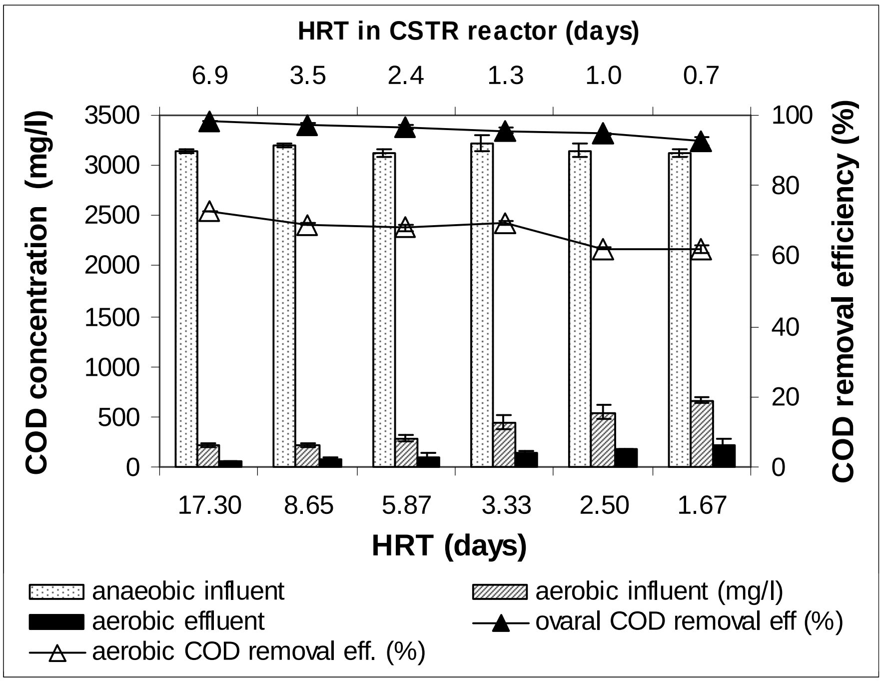


Figure 4.60 COD removal efficiencies in aerobic CSTR reactor and anaerobic (AMBR)/aerobic (CSTR) sequential reactor system.

4.2.3.2.7 Variation Specific Methanogenic Activity (SMA) and F/M ratios in AMBR at Different HRTs. Figures 4.61 and 4.62 show the SMA and F/M ratio variations in AMBR reactor during the continuous operation. As shown in Figure 4.61, SMA decreased from 0.42 to 0.13 g COD-CH_4/gVSS day as the HRT decreased from 10.38 days to 1day. SMA was high at HRTs such as 10.38 days and 5.19 days. SMA values decreased when HRT decreased. A strong linear correlation between HRT and SMA was observed ($R^2 = 0.93$; d.f. = 5, F = 24.13, p = 0.008)**.** This shows that the methanogenic activity decreased with decreasing HRTs. Low HRTs does not provide enough time to increase the activity for methanogens. However the F/M ratios increased with decreasing of HRT (See Fig. 4.62). F/M ratio increased from 0.004 to 0.05 day^{-1} as the HRT decreased from 10.38 days to 1 day. Increase of F/M ratio depends to higher OLRs or lower HRTs applied to the reactor. Furhermore, high VSS concentration in AMBR decrease the F/M ratios at high HRTs and at low HRTs, the VSS could be released from the AMBR reactor effluent and cause increases of F/M ratio.

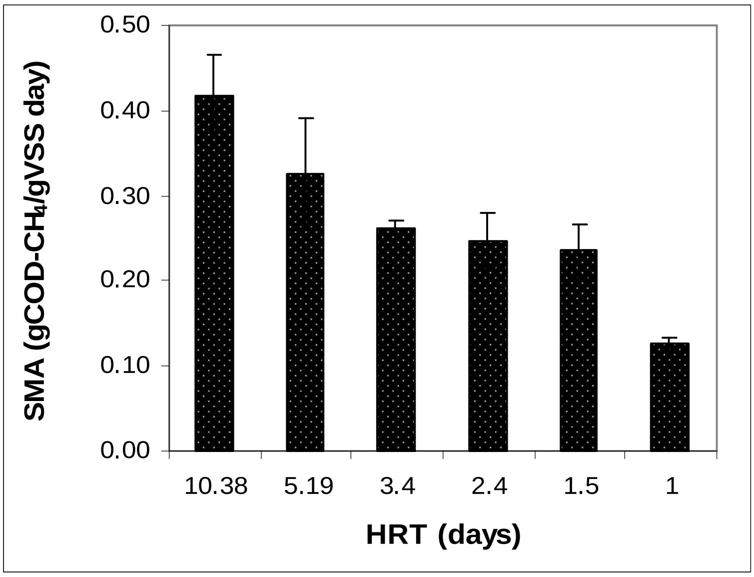


Figure 4.61 SMA values in AMBR at different HRTs.

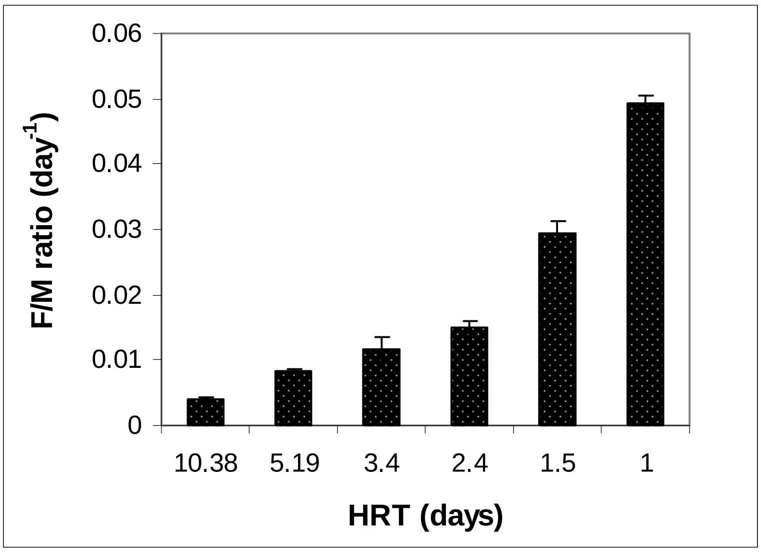


Figure 4.62 F/M ratio in AMBR at different HRTs.

4.2.4 The removal of NB in ABR and Sequential ABR/CSTR Reactor System

4.2.4.1 Effect of Increasing NB Loading Rates on Performance of ABR Reactor

4.2.4.1.1 Effect of Increasing NB Loading Rates on the COD and NB Removal Efficiencies in ABR Reactor. The operation of the ABR was started at an influent NB concentration of 20 mg/l and increased step to step from 30, 55, 130, 150, 170, 210, 300, 450, 550 to 700 mg/l corresponding to NB loading rates of 1.93, 5.3, 12.52, 14.4, 20.2, 28.9, 43.3, 52.9 and 67.4 g/m^{3}day at a HRT of 10.38 days. Figure 4.63 shows the COD removal efficiency at increasing NB loading rates in ABR. The COD removal efficiency was 92% at a NB loading rates of 1.93 g/m^{3}day. The COD removal efficiencies decreased with decreasing NB loading rates. Optimum NB loading rate was found as 12.5 g/m^{3}day corresponding to NB concentration of 130 mg/l, for maximum 92% COD and %100 NB removal efficiencies. The COD removal efficiency was 91% at NB loading rate of 20.23 g/m^{3}day correspond to nitrobenzene concentration of 210 mg/l. After 20.23 g/m^{3}day of NB loading rate, the COD removal efficiencies decreased from 91% to 80%. COD removal efficiency was 80% at NB loading rate of 67.4 g/m^{3}day and at NB concentrations of 700 mg/l. The effluent COD concentration was 795 mg/l. High NB loading rates can not be tolerated by the methanogen *Archaea* and COD could not be degraded. Thus, high COD concentrations measured in the effluent of ABR.

The effects of increasing NB loading rate on the NB removal efficiencies are shown in Figure 4.64. NB removal efficiencies were approximately 100 % at all NB loading rates. The effluent NB concentrations were zero at all NB loading rates. For the maximum COD and NB removal efficiencies (E=91-92% and 100%, respectively) the optimum NB loading rates and NB concentrations were found between 1.93-20.93g/m^{3}day and 20-210 mg/l, respectively.

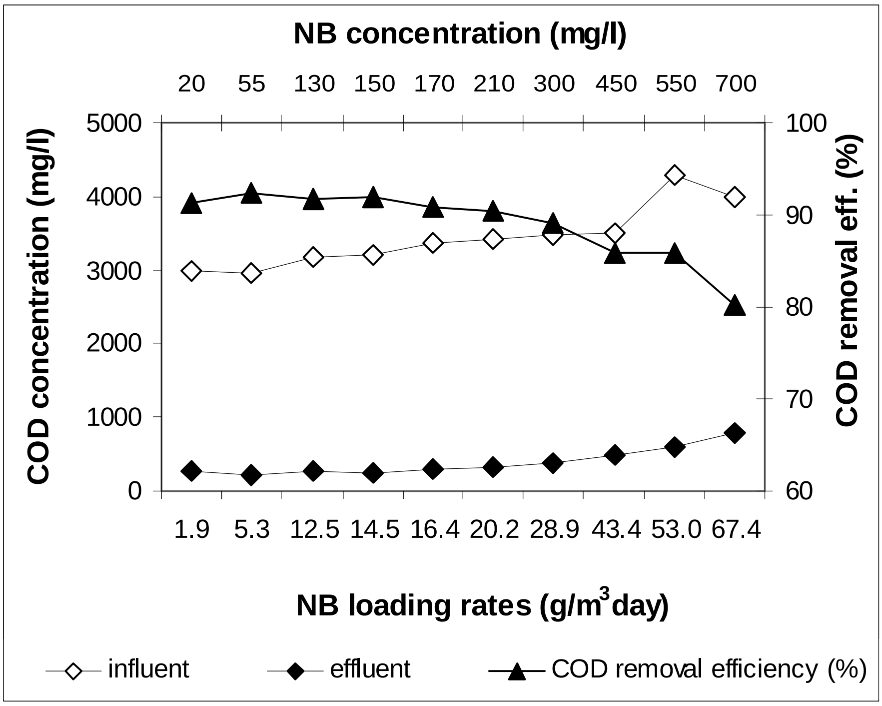


Figure 4.63 Effect of NB loading rate on COD removal efficiency in ABR.

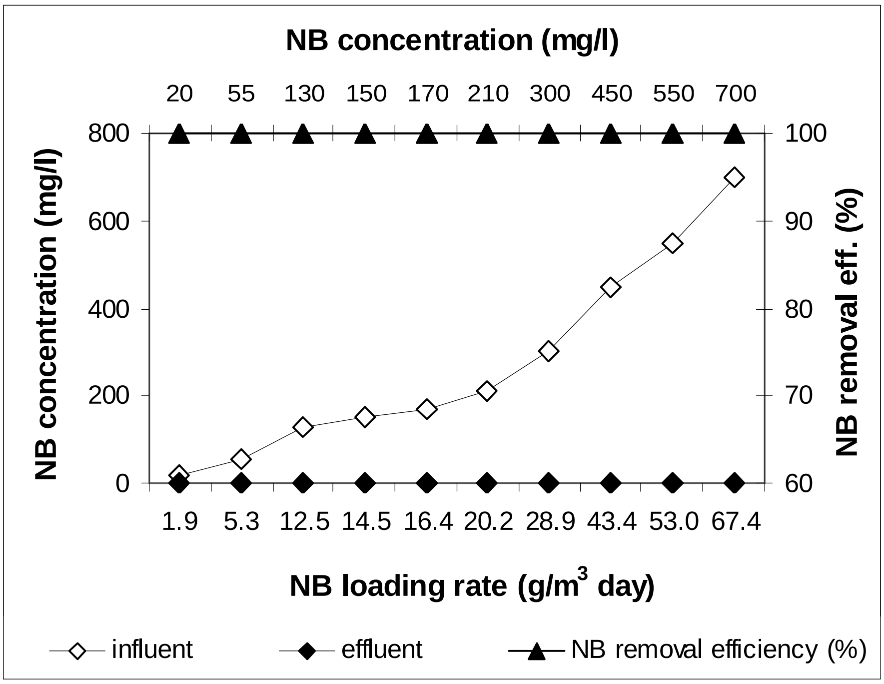


Figure 4.64 Effect of NB loading rate on NB removal efficiencies in ABR.

4.2.4.1.2 Effects of Increasing NB Loading Rates on the Total and the Methane Gas Production in ABR Reactor. Figure 4.65 shows the effect of NB loading rate on gas production and methane percentage. The daily total gas, methane gas productions and % methane percentage were about 1300 ml/day, 3300 ml/day and 50 %, respectively, until a NB loading rate of 20.23 g/m^{3}day. The maximum total gas, methane gas productions and methane percentage were found in the aforementioned NB loading rates. After this loading rate, the daily total gas, methane gas productions and methane percentage decreased. Total gas, methane gas production rates and methane percentage were found as 1890 ml/day, 820 ml/day and 38% at maximum NB loading rate of 67.4 g/m^{3}day. This indicated the inhibition effect of NB on methane *Archaea* at aforementioned NB loading rates.

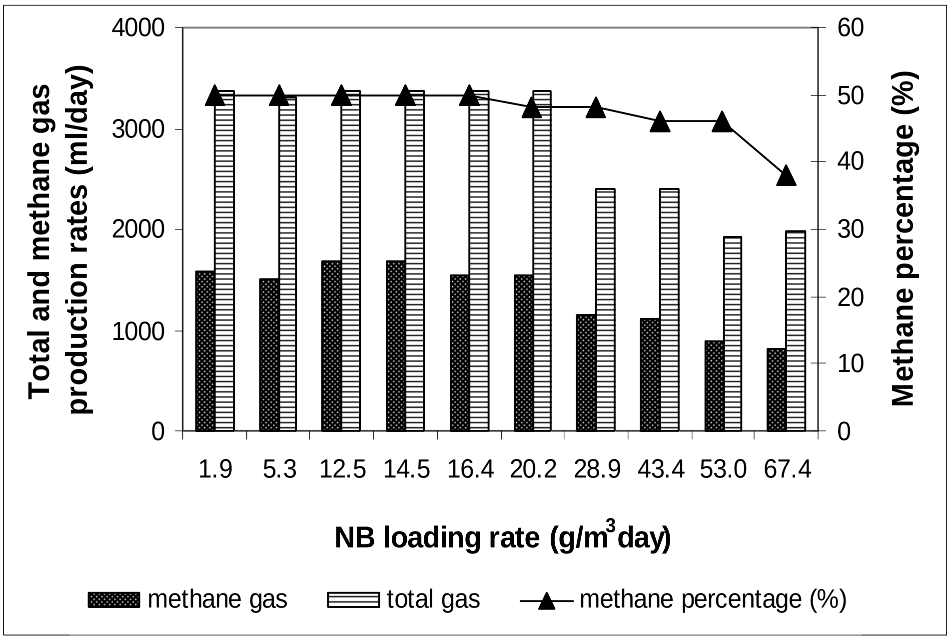


Figure 4.65 Effect of NB loading rates on total gas, methane gas productions and methane percentage.

4.2.4.1.3 Variations of COD, NB Removal Efficiencies in the Compartments of the ABR at Increasing NB Loading Rates. Figure 4.66 shows the COD removal efficiencies in the compartments of ABR. As shown in Fig. 4.66, the COD removal efficiencies were higher in the first compartment than the other two compartments. The COD removal efficiencies were found around 80% in the first compartment at all NB loading rates. The COD removal efficiencies were 10-20% in the second compartment, 10-30% in the third compartment at all NB loading rates. The COD concentration was 1200 mg/l in initial compartment, 950 mg/l in second compartment and 795 mg/l in third compartment at high NB loading rate such as 67.4 g/m^{3}day. Table 4.13 shows the NB concentration and removal efficiencies in the compartments of ABR. The NB removal efficiencies were higher in initial compartment than the other compartments. The majority of NB removed in the first compartment (E=99-100%) at all NB loading rates. This can be explained by the conversion of NB to aniline at very short times. Peres, Naveau, & Agathos, (1998) found that 10 mg/l of NB was biodegraded to its corresponding aromatic amine (aniline) less than 20 h. The NB concentration was around 1.5-2.5 mg/l at high NB loading rates such as 53 and 67.4 g/m^{3}day in the first compartment. However the NB concentrations in compartment 2 and 3 were approximately 0 mg/l at all NB loading rates. The NB concentration was 2.5 mg/l in initial compartment, 0 mg/l in second compartment 0 mg/l in third compartment at high NB loading rate such as 67.4 g/m^{3}day. These results showed the positive effect of compartmentalisation of ABR reactor based on COD and NB removal efficiencies. Figure 4.67 shows the HPLC chromatogram of sample taken from the anaerobic effluent at a maximum influent NB concentration of 700 mg/l. No NB peak was observed in HPLC chromatogram. This showed that NB was biodegraded completely to aniline even at high NB concentration such as 700 mg/l in ABR reactor. The aniline peak was appeared at retention time of 3.52 min.

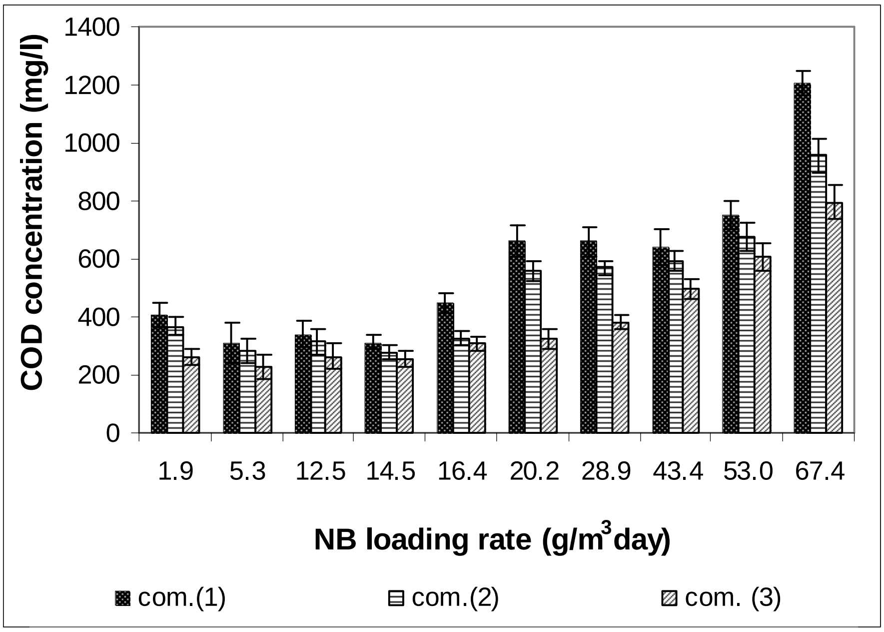


Figure 4.66 The COD concentration variations in compartments of ABR.

Table 4.13 Variations of NB concentration in compartments of ABR.

NB loading rate (g/m^3day)	**Influent NB con. (mg/l)**	**compartment 1 (mg/l)**		**compartment 2 (mg/l)**		**compartment 3 (mg/l)**	
		NB con. (mg/l)	**NB removal eff. (%)**	**NB con. (mg/l)**	**NB removal eff. (%)**	**NB con. (mg/l)**	**NB removal eff. (%)**
1.9	30	0.0	100	0.0	100	0.0	100
5.3	54	0.0	100	0.0	100	0.0	100
12.5	130	0.0	100	0.0	100	0.0	100
14.5	150	0.0	100	0.0	100	0.0	100
16.4	170	0.0	100	0.0	100	0.0	100
20.2	210	0.0	100	0.0	100	0.0	100
28.9	300	0.0	100	0.0	100	0.0	100
43.4	450	0.0	100	0.0	100	0.0	100
53.0	550	1.6	99	0.0	100	0.0	100
67.4	700	2.54	99	0.0	100	0.0	100

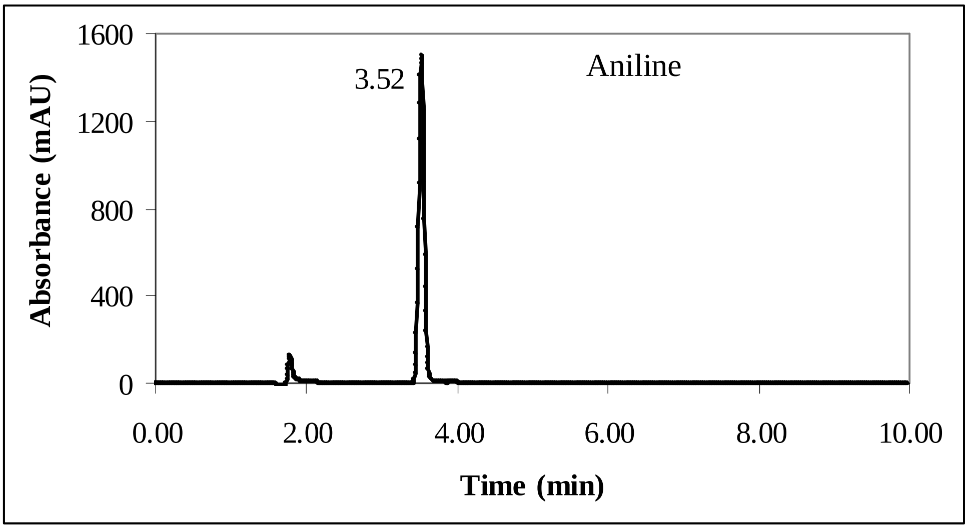


Figure 4.67 HPLC chromatogram of ABR effluent at NB concentration of 700 mg/l.

4.2.4.1.4 The Variation of pH, TVFA, Bicarbonate Alkalinity (Bic.Alk.) and TVFA/Bic.Alk. ratio in Compartments of the ABR at Increasing NB Loading Rates. Figure 4.68 shows the variations in pH, TVFA, bicarbonate alkalinity and TVFA/Bic.Alk. ratio in compartments of ABR reactor at increasing NB concentrations. As shown in Figure 4.68 (a), pH values in the effluent and in the compartments of ABR did not vary. The pH values were between 7.2 and 7.9, as optimum pH values, in the compartments and in the effluent samples at increasing NB loading rates. pH values was lower in the first compartment than the other compartments due to higher TVFA in the first compartment (See Fig. 4.68 (b)). The TVFA concentrations increased from 32 mg/l to 160 mg/l in the first compartment as the NB loading rate increased from 1.93 to 67.4 g/m^{3}day. TVFA concentration was zero in the effluent with the exception 67.43 g/m^{3}day of NB loading rate. The TVFA concentration was measured as 32 mg/l in the effluent samples of ABR reactor for a NB loading rate of 67.43 g/m^{3}day. The Bic.Alk. concentrations were lower in the first compartment than the others compartment. This can be explained higher TVFA concentration in the first compartment (See Fig. 4.68 (c)). The Bic.Alk. of 3400 mg/l in the influent decreased to 2900 mg/l in the first compartment of ABR at NB loading rate of 67.4 g/m^{3}day. This shows the buffer effect of Bic.Alk. in ABR reactor. Fig. 4.68 (d) shows the TVFA/Bic.Alk. ratio. These ratios varied between 0.01 and 0.03 in compartment 1 until a NB loading rate of 53 g/m^3.day in ABR. TVFA/Bic.Alk. ratios were 0.03 at NB loading rates of 53-67.4 g/m^{3}day in the first compartment. These ratios were between 0.2-0.21 in the second compartments for the aforementioned NB loading rates. These results indicated that ABR reactor was stable at increasing NB loading rates because the TVFA/Bic.Alk. ratios in the effluent and in the compartments were lower than 0.04.

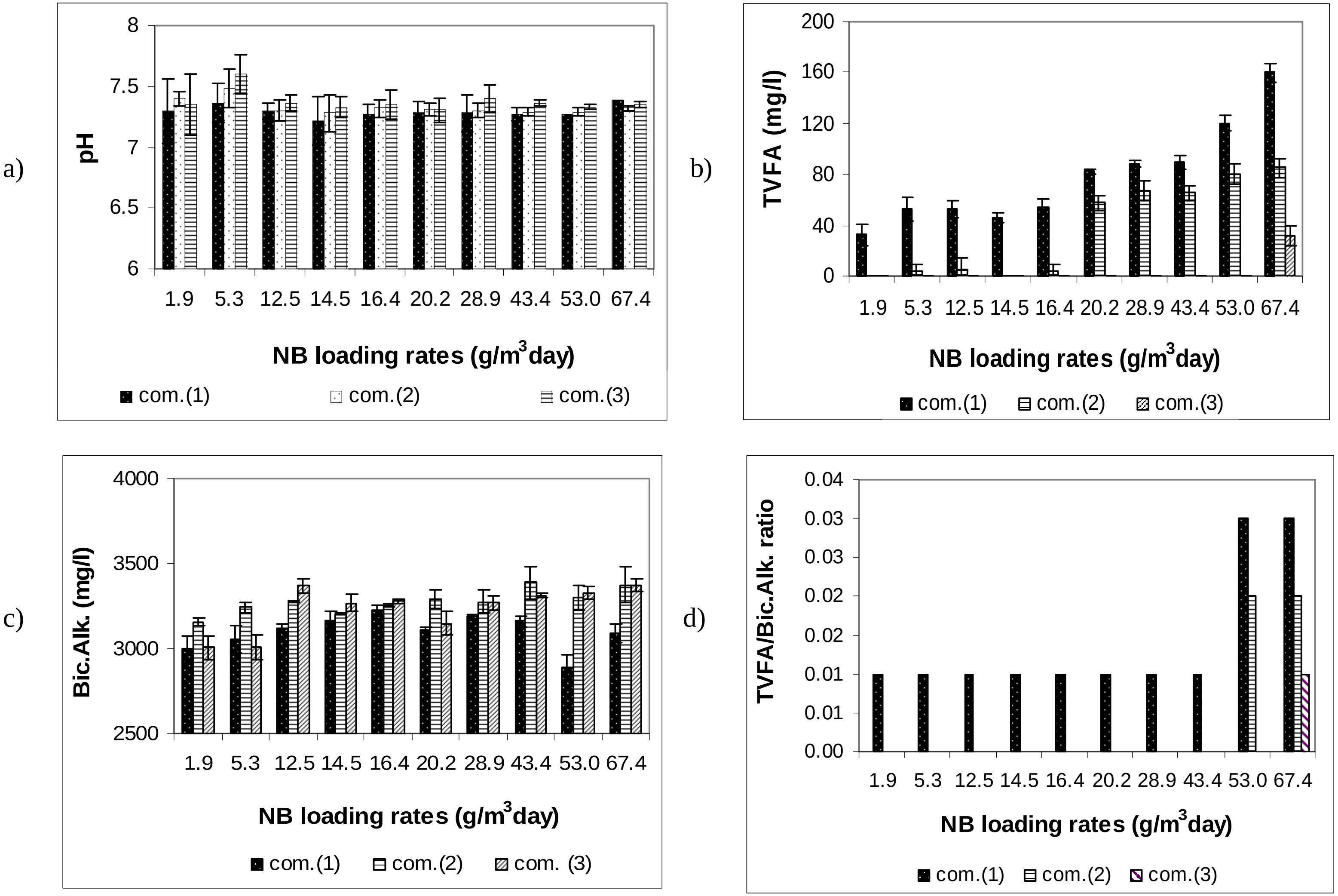


Figure 4.68 The variations of pH (a), TVFA (b), Bic.Alk. (c) and TVFA/Bic.Alk. ratio (d) in ABR reactor at increasing NB loading rates.

4.2.4.1.5 Performance of Aerobic CSTR Reactor and Treatment Efficiencies of Sequential Anaerobic (ABR)/ Aerobic (CSTR) Reactor System. Figure 4.69 shows the aniline concentration in the effluent of ABR and CSTR reactor and aniline removal efficiency in CSTR at increasing NB loading rates. As shown in this Figure, the effluent aniline concentration increased as the influent NB concentration increased (R^2 = 0.98; d.f. = 9, F = 174, p = 1.04E-6). Aniline concentration in the effluent increased from 14 mg/l to 286 mg/l when the NB loading rate in the influent increased from 1.7 g/m^3day to 67.4 g/m^3.day. This shows that NB was biodegraded to aniline under anaerobic conditions. Aniline removal efficiencies in CSTR were 100% until a NB loading rate of 28.9 g/m^3day. After this NB loading rate, the aniline removal efficiencies decreased from 100% to 88% at aNB loading rate of 67.4 g/m^3day. The aniline removal efficiency was 100% at low NB loading rate and degraded completely at NB loading rates of 28.9 g/m^3.day in aerobic reactor.

Figure 4.70 shows the COD removal efficiency of aerobic reactor and total COD removal efficiency in sequential anaerobic/aerobic reactor system. The COD and aniline remaining from the ABR were removed in aerobic reactor. COD removal efficiencies varied between 62 % and 87 % for NB loading rates of 1.9 g/m^3day and 67.4 g/m^3day corresponding to NB concentration of 20 and 700 mg/l in aerobic CSTR. The COD concentration in the effluent of CSTR was 105 mg/l at NB loading rate of 67.4 g/m^3.day. Total COD removal efficiencies varied between 95% and 99% in sequential ABR/CSTR reactor system.

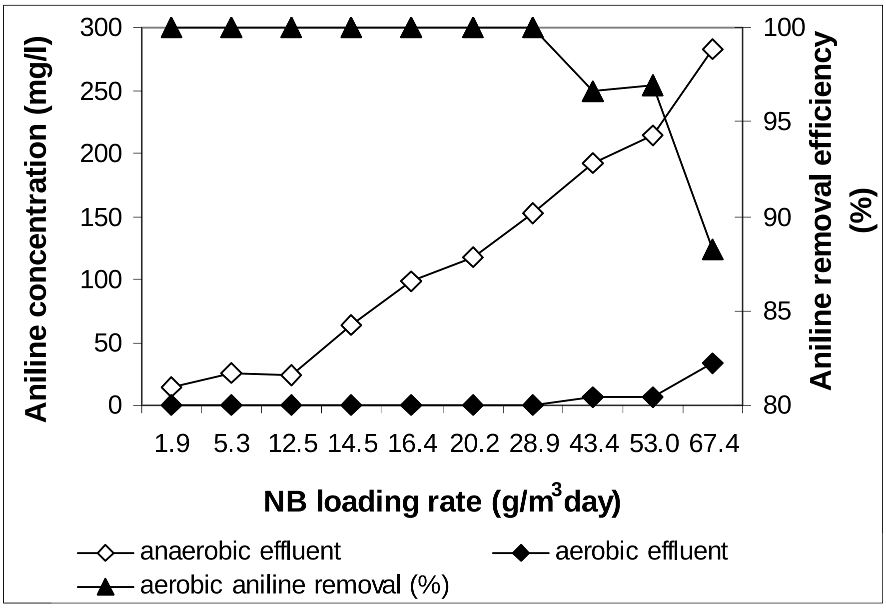


Figure 4.69 Aniline concentrations in the effluent of ABR and CSTR reactor and aniline removal efficiency in CSTR at increasing NB loading rates.

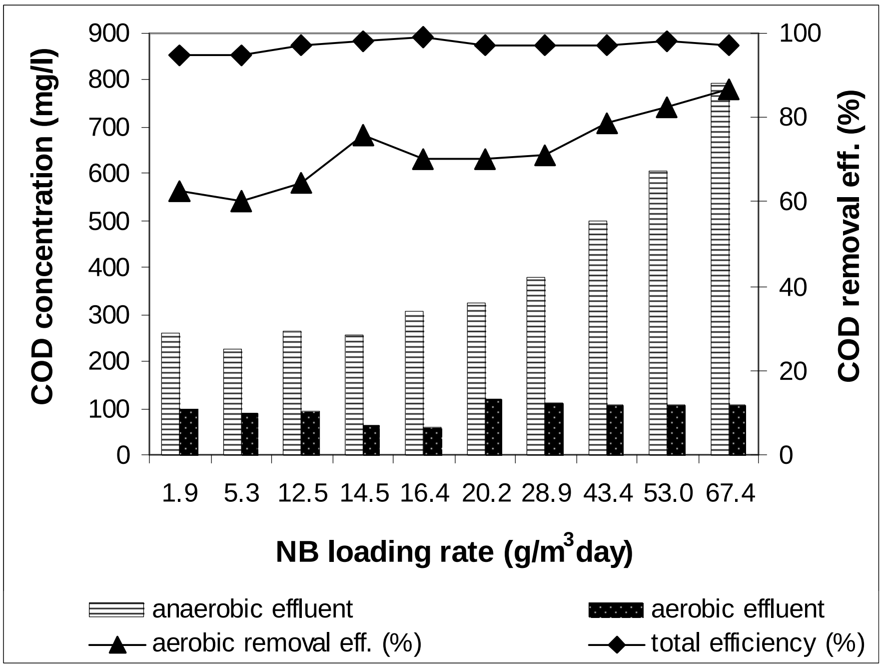


Figure 4.70 COD removal efficiency in aerobic CSTR reactor and total COD removal efficiency in ABR/CSTR reactor system.

4.2.4.2 Effect of Hydraulic Retention Time (HRT) on the Performance of ABR Reactor

4.2.4.2.1 Effects of HRT on COD and Nitrobenzene Removal Efficiencies in Anaerobic ABR Reactor. The effect of HRT on the COD and NB removal efficiencies in ABR are shown in Figure 4.71. The influent COD and NB concentrations were kept constant at 3000 mg/l and 130 mg/l, respectively, through continuous operation. 94% COD and 99.9% NB removal efficiencies were obtained at a HRT of 10.38 days. NB removal efficiency were 99.9 % through all HRTs. The COD removal efficiency decreased from 94% to 86% when the HRT decreased from 10.38 days to 1 day. The COD removal efficiency was found as 86 % at minimum HRT of 1 day. The maximum COD removal efficiencies were found between 94-93 % at HRTs varying between 10.38 days and 2.5 days. As the HRT decreased the COD removal efficiency also decreased. However no variation in NB removal efficiency was observed. The NB removal was 100% through all HRTs. NB was completely removed even at very short HRTs such as 1 day in ABR reactor. NB concentrations were approximately zero in the effluent of ABR at all HRTs. The reason of low COD removal efficiency was the accumulation of intermetabolic aromatic amines generated from the degradation of NB, which measured as COD.

Figure 4.72 shows the HPLC chromatogram for the effluent of ABR at a HRT of 1 day. This figure clearly demonstrates that NB was completely degraded to aniline through anaerobic treatment. The peak for NB standard was obtained at a retention time of 7.72 min (Data not shown). This retention time was not shown in chromatograms of AMBR effluent samples. The retention time for effluent samples was 3.44 min., indicating that NB was degraded to aniline since the peak in the aniline standard was obtained at a retention time of 3.44 min.

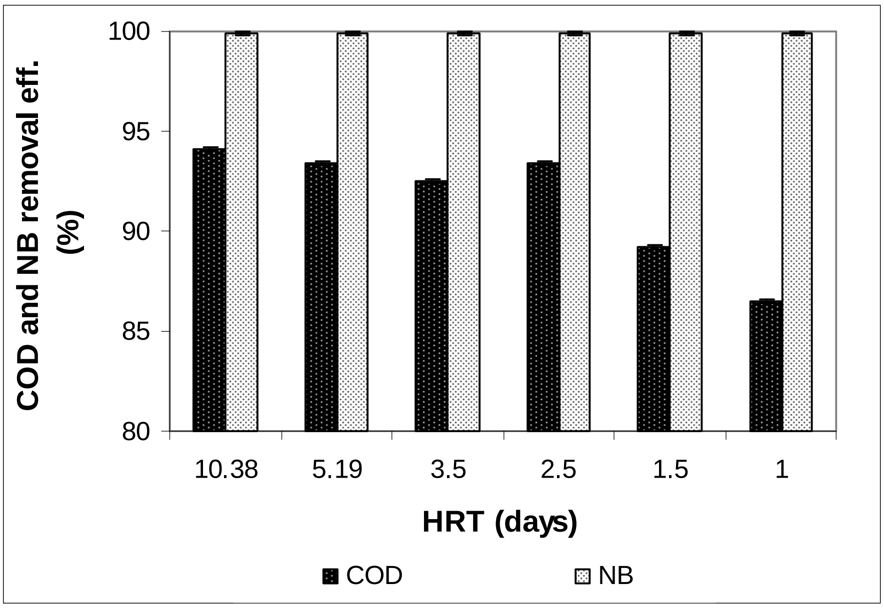


Figure 4.71 The effect of HRT on the COD and NB removal efficiencies in ABR.

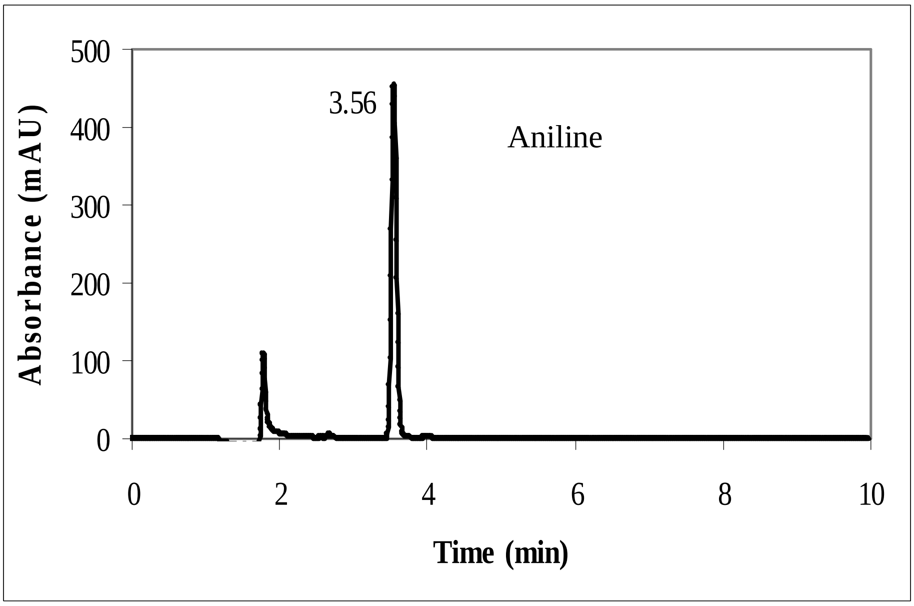


Figure 4.72 HPLC chromatogram in the effluent of ABR at 1 day of HRT.

4.2.4.2.2 Effects of HRT on the Total and the Methane Gas Productions in ABR. Figure 4.73 shows the effect of NB loading rate on gas production and methane percentage. The daily total and methane gas productions increased from 2.8 l/day to 17.2 l/day and from 1.2 l/day to 5.1 l/day as HRT decreased from 10.38 days to 1 day, respectively. The maximum total gas, methane gas productions were found at HRT of 1 day. High total and methane gas production could be explained to high OLRs at low HRTs resulting in high gas productions. Methane gas percentages decreased from 43% to 29% as the HRT decreased from 10.38 days to 1 day, respectively. Methane percentage was 29% at this HRT. Low methane percentages can be explained by the partial dominancy of acidogenesis compared to methanogenesis at low HRTs in ABR. Furthermore, the high NB loading rates at low HRTs varied the gas composition (Speece, 1996). As shown in Figure 4.74, it can be seen that the methane yield (Y) decreased with decreasing HRT. Methane yield (Y) decreased from 0.15 to 0.062 m^3CH_4/kgCOD removed when the HRT was decreased from 10.38 to 1 day. These results exhibit similarities with the data reported by Kuscu & Sponza, (2006). They found that the methane yield decreased from 0.16 to 0.07 m^3 CH_4/kg COD removed as the HRT decreased from 10.38 days to 1 day.

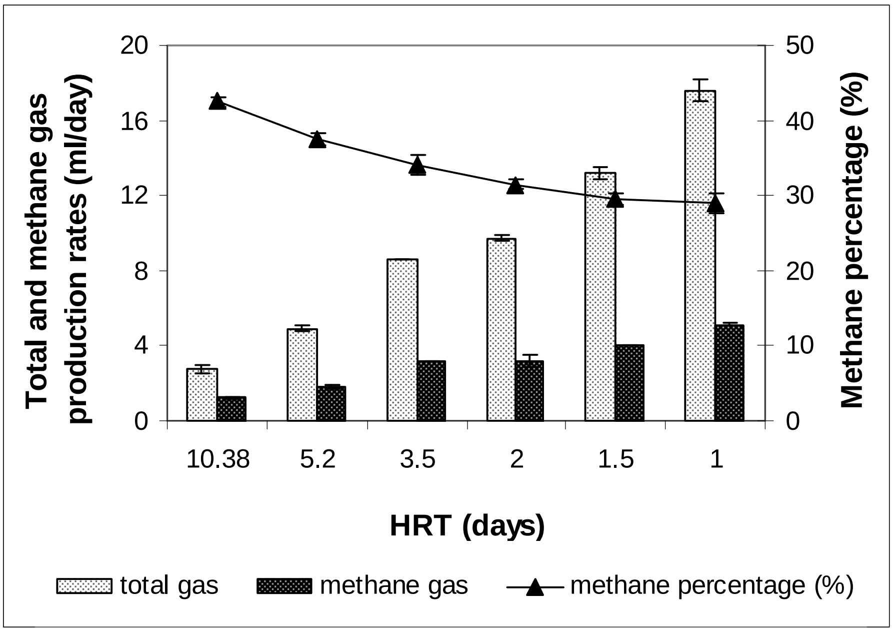


Figure 4.73 The effect of HRTs on gas production and methane percentage in ABR.

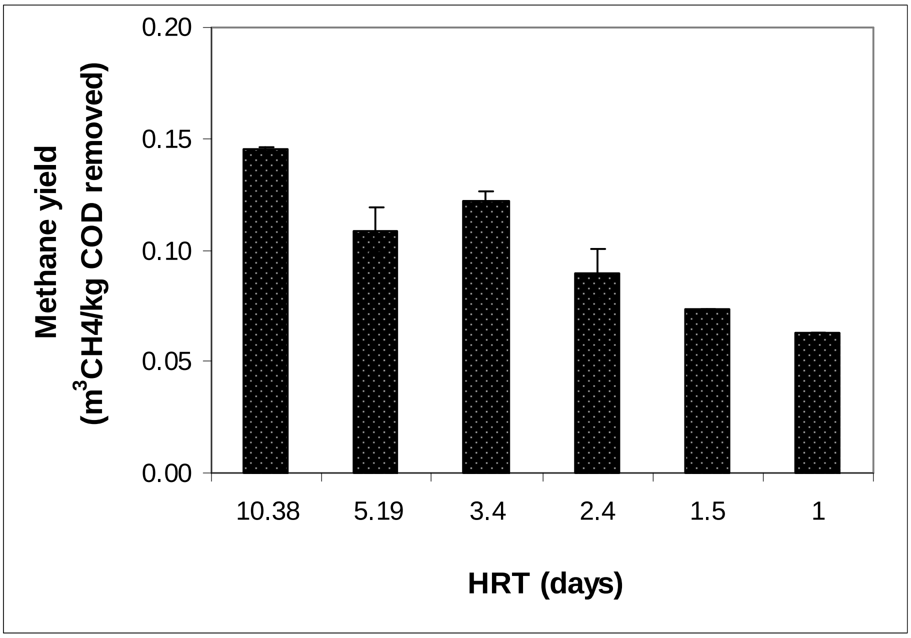


Figure 4.74 The effect of HRTs on methane yield (Y) in ABR.

4.2.4.2.3 Effect of Compartments on COD and NB Removal Efficiencies in ABR. Table 4.14 shows the COD variations and COD removal efficiencies in all compartments of ABR. The majority of the influent COD was removed in compartment 1. The COD removal efficiencies in first compartment decreased from 93 % to 70% as the HRT decreased from 10.38 days to 1 day. However COD removal efficiency increased from %14 to 34% in the second compartment with decreasing of HRT. This could be explained with compartmentalised structure of ABR. The first compartment of an ABR may act a buffer zone to shock loading and toxic material in the feed, and thus allows the later compartments to be loaded with a relatively harmless (Uyanik, Sallis, & Anderson, 2002). A few amount of COD removal (4–19%) were measured in compartment 3.

Table 4.15 shows NB variation and NB removal efficiencies in compartments of ABR. NB removal efficiencies were 100% in the first compartment of ABR at all HRT. It is important to activated population the sensitive methanogenic bacteria in the compartment 2. In the compartmentalised reactors, acidogenic bacteria are dominant in the first compartment, while methanogenic bacteria are dominant in the subsequent compartments (Barber & Stuckey, 1999).

Table 4.14 Variations of COD concentration and COD removal efficiency in compartments of ABR.

HRT (days)	**COD concentration (mg/l) and COD removal efficiency (%) in compartments**					
	compartment 1		**compartment 2**		**compartment 3**	
	Con. (mg/l)	**Efficiency (%)**	**Con. (mg/l)**	**Efficiency (%)**	**Con. (mg/l)**	**Efficiency (%)**
10.38	228±41	93±1	196±3.5	14±1	188±14	4±1
5.19	325.5±8	90±2	262±7.8	19±0.2	211±23	19±1
3.5	297±11	91±1	265±16	19±1	250±5	6±1
2	380.5±57	88±2	227±13	40±2	210±6	7±1
1.5	624.5±35	80±2	372±70	40±2	345±23	7±1
1	1010±65	70±2	668±25	34±2	548±31	18±1

Table 4.15 Variations of NB concentration and removal efficiency in compartments of ABR.

HRT (days)	**Influent NB con. (mg/l)**	**compartment 1 (mg/l)**		**compartment 2 (mg/l)**		**compartment 3 (mg/l)**	
		NB con. (mg/l)	**NB removal (%)**	**NB con. (mg/l)**	**NB removal (%)**	**NB con. (mg/l)**	**NB removal (%)**
10.38	130±2	0.0	100	0.0	100	0.0	100
5.19	130±1	0.0	100	0.0	100	0.0	100
3.5	130±1	0.0	100	0.0	100	0.0	100
2	130±1	0.0	100	0.0	100	0.0	100
1.5	130±1	0.0	100	0.0	100	0.0	100
1	130±1	0.0	100	0.0	100	0.0	100

4.2.4.2.4 Effects of HRTs on pH, TVFA and Bicarbonate Alkalinity Variations in the Effluent of the ABR. Figure 4.75 shows the pH, TVFA, Bic.Alk. variations and TVFA/Bic.Alk. ratio in the effluent of ABR reactor at different HRTs. The effluent pH values remained stable between 7.2 and 7.4 in ABR at all HRT (See Figure 4.75). TVFA concentration in the effluent of ABR increased when the HRT decreased (R^2=0.96, df=5, p=0.002, F=55.7). When HRT decreased from 10.38 days to 1 day, TVFA concentration increased from 0 to 480 mg/l. This could be explained by the low COD removal since there were some difficulties in biotransformation of intermediates of glucose, namely acetic and propionic acids which were measured as TVFA.

However, the effluent pH values were between optimum values. This was succeeded with 5000 mg/l Bic.Alk. added in the feed. Figure 4.76 shows the variations in Bic.Alk. and TVFA/Bic.Alk. ratio variations in the effluent of ABR. The Bic.Alk. concentrations decreased from 3078 mg/l to 2560 mg/l as HRT decreased from 10.38 days to 1 day. This shows that Bic.Alk. used to adjust the pH in the anaerobic reactor since TVFA was high at low HRTs in the anaerobic reactor. TVFA/Bic.Alk. ratio increased as depending on increasing of TVFA in the effluent of ABR with decreasing HRT. However, TVFA/Bic.Alk. ratios were below 0.3 at all HRTs. This shows the stability of ABR reactor (Behling et al., 1997).

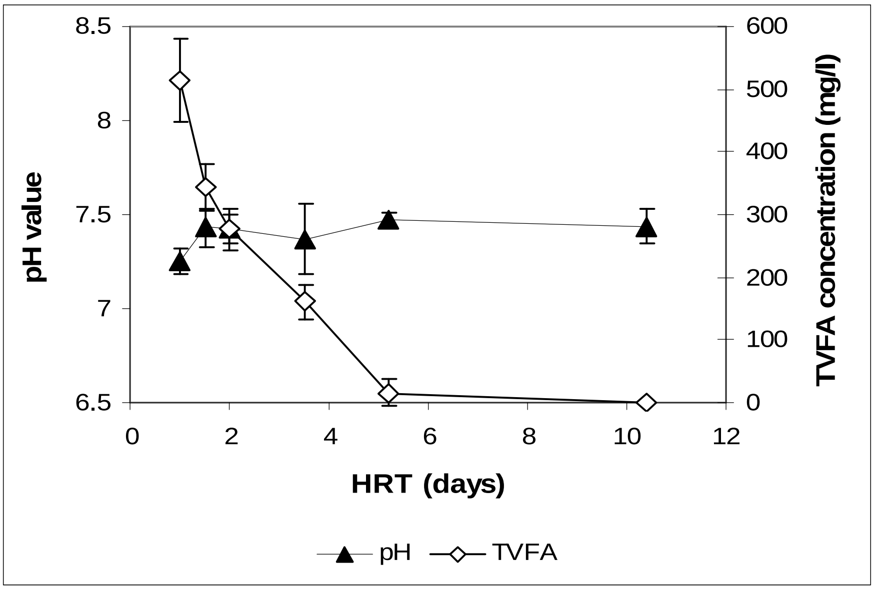


Figure 4.75 Variations of pH and TVFA in the effluent of ABR reactor at different HRTs.

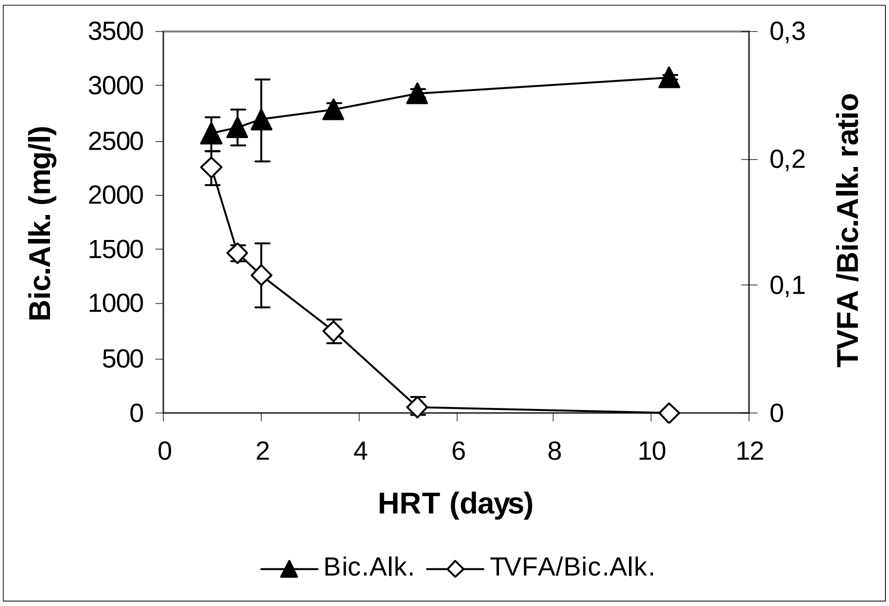


Figure 4.76 Variations of Bic.Alk. and TVFA/Bic.Alk. ratios in the effluents of ABR reactor at different HRTs.

4.2.4.2.5 Performance of Sequential Anaerobic ABR/Aerobic CSTR Reactor System. Figure 4.77 shows the overall COD removal efficiency in anaerobic/aerobic sequential reactor system. The effluent of ABR was used as the influent of CSTR reactor and anaerobic/aerobic sequential reactor system was constituted. COD removal in the total system varied between 96% and 98% at all HRTs. The effluent COD concentration increased from 50 to 147 mg/l as the HRT decreased from 17.3 to 1.67 days, respectively, in anaerobic/aerobic system. The majority of NB was removed in anaerobic reactor into anaerobic/aerobic sequential reactor system. 100 % NB removal was observed in anaerobic reactor (See Figure 4.71). 96-98% COD removal and 100% NB removal efficiencies was measured at HRTs varied between 17.3 and 1.67 days in sequential anaerobic/aerobic system. These results were higher than the results reported by Aziz, Ng, & Zhou (1994). Aziz, Ng & Zhou (1994) observed that COD and NB removal efficiencies were approximately 70% and 99%, respectively as the HRT decreased from 24 h to 8 h.

Figure 4.78 shows the aniline removal efficiency in aerobic reactor. 70-80 mg/l of aniline produced in the anaerobic step was removed with removal efficiencies variying between 50% and 100% depending to HRT in CSTR reactor. Aniline removal efficiencies were 100% until a HRT of 3.5 days in CSTR. After this HRT, aniline removal efficiencies decreased rapidly. Aniline removal efficiency was 50% in the aerobic CSTR reactor at a HRT of 0.7 day (17 h). Lower aniline removal efficiency was observed at lower HRTs. Peres, Naveau, & Agathos (1998) found that 20 mg/l of aniline was completely biodegraded at 11 h. This exhibits similar results with our date. Lower aniline removal was observed at lower HRTs.

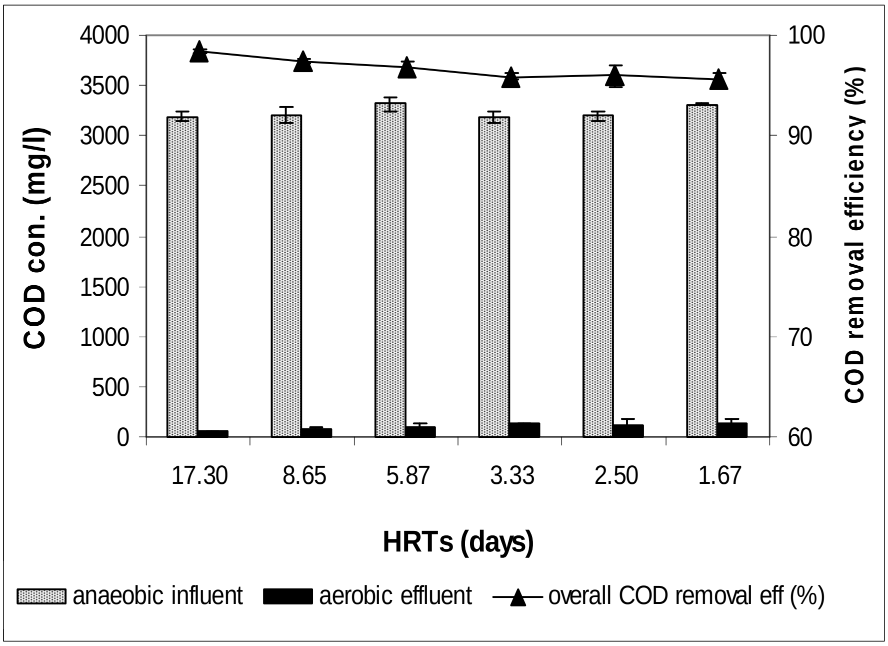


Figure 4.77 Total COD removal efficiency in anaerobic (ABR)/aerobic (CSTR) sequential reactor system.

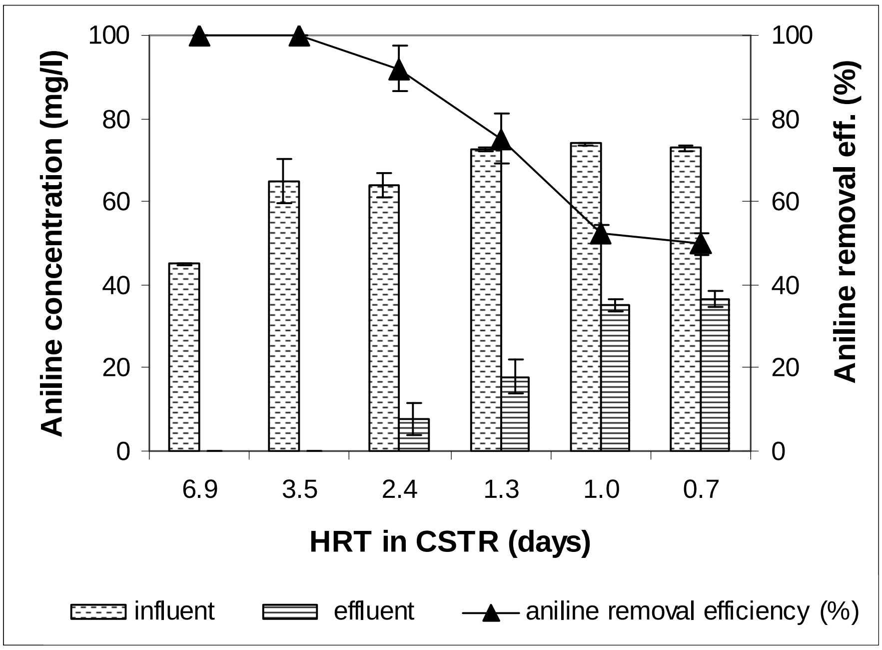


Figure 4.78 Aniline removal efficiency in aerobic CSTR reactor.

4.2.4.2.6 Variations in Specific Methanogenic Activity (SMA) and F/M Ratios in ABR at Different HRTs. Figures 4.79 and 4.80 show the variations of SMA and F/M ratio in ABR reactor during the continuous operation. As shown in Figure 4.79, SMA decreased from 0.39 to 0.19 gCOD-CH_4/gVSS day when the HRT decreased from 10.38 days to 1 day. SMA was high at HRTs such as 10.38 days and 5.19 days. SMA values decreased when the HRT lovered (R^2 = 0.94; d.f. = 5, F = 33.4, p = 0.004). This shows that methanogenic activity decreased with decreased HRTs. This result is comparable with those obtained by (Fang, Chen, Li, & Chui, 1996) in an UASB reactor treating only phenol (SMA=0.98 g CH_4COD/g VSS d) and acetate (0.64 g CH_4 COD/g VSS d).

Figure 4.80 shows the variations in F/M ratio in ABR at different HRTs. The F/M ratio increased with decreasing HRT (See Fig. 4.80). F/M ratio increased from 0.006 to 0.05 g COD/gTSS day as the HRT decreased from 10.38 days to 1 day. Increase of F/M ratio depends on increase of OLRs. A strong linear correlation between OLR and F/M ratio was observed (R^2 = 0.98; d.f. = 5, F = 80.27, p = 0.00086). A strong linear correlation between HRT and F/M was not observed (R = 0.76; d.f. = 5, F =

5.6, p = 0.07). Furthermore, F/M ratio variations depended on very much OLR (R^2 = 0.98; d.f. = 5, F = 80.27, p = 0.00086) as compared to VSS concentration in reactor (R^2=0.14, f=0.57, p=0.8, df=0.09). Maximum VSS concentration in ABR was found as 80 g/l at a HRT of 3.4 days. After this HRT, VSS concentrations in ABR decreased from 80 g/l to 63 g/l at HRTs varied between 2.4 and 1 day. This can be explained to high VSS washouth from reactor to efflunt at high OLRs (lower HRTs).

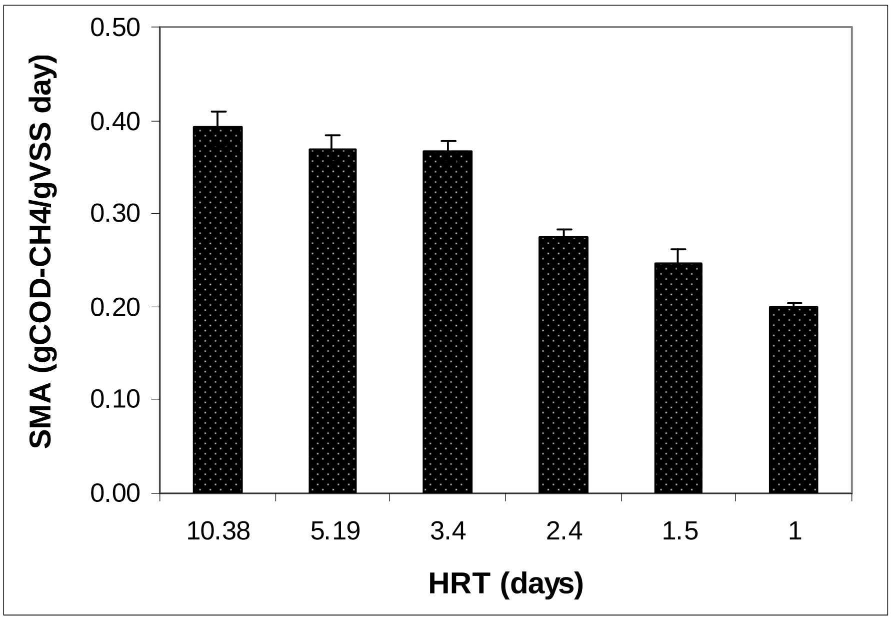


Figure 4.79 SMA values in ABR at different HRTs.

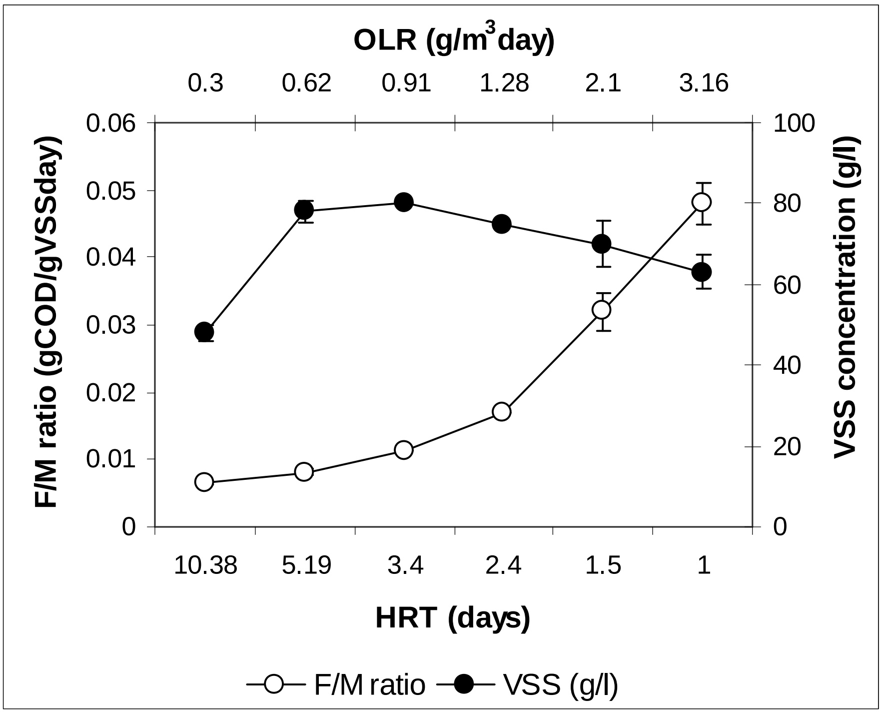


Figure 4.80 Variations of F/M ratio in ABR at different HRTs.

4.2.4.2.7 Assessment of Toxicity in Sequential Anaerobic ABR/Aerobic CSTR Reactor System. In this study, toxicity of effluent of ABR and aerobic (CSTR) reactor was determined by bioluminescence test using *Photobacterium phosphoreum* bacteria (LCK 480). Table 4.16 shows the inhibition percentage of samples taken from the influent synthetic wastewater containing a NB concentration of 100 mg/l, anaerobic and aerobic effluent of ABR at incubation times of 5th,15th, 30th min. at a HRT of 3.5 days.

The toxicity results of the test were obtained for short time (maximum 30 min). As shown in Table 4.16, the inhibition percentage (H) of influent was founded as 87 % at a incubation time of 30 min. This result showed that wastewater containing NB of 100 mg/l was toxic (Lange, 1994). After anaerobic treatment, the effluent toxicity decreased to 48 % at incubation time of 30 min.

This shows that the effluent of anaerobic reactor was less toxic (H=48%) than the feed containing NB (Lange, 1994). In other words anaerobic conditions constituted at less toxic intermediate products. After aerobic treatment, toxicity decreased from 48 % to 18 % in aerobic effluent at an incubation time of 30 min. The aerobic reactor effluent was less toxic (Lange, 1994). It was observed that the toxicity of influent of anaerobic reator were high, whereas after anaerobic/aerobic treatment toxicity was decreased.

Table 4.16 Lumistox toxicity values in sequential ABR/ CSTR reactor system (HRT=3.5 days).

Inhibition H (%)	**Time (min.)**	**Anaerobic Influent**	**Anaerobic effluent**	**Aerobic effluent**
H_5	5	83	36	34
H_{15}	15	85	45	25
H_{30}	30	87	48	18

4.2.4.2.8 General Discussion. Table 4.17 shows the COD and p-NP removal efficiencies, total and methane gas production and methane percentage and effluent pH, TVFA, Bic.Alk. concentrations and TVFA/Bic.Alk. ratio in effluent of AMBR and ABR reactor treating NB at increasing NB loading rate and different HRTs. As seen in Table 4.17, NB removal efficiencies were 100% in every two reactors. This showed that NB transformed completely to aniline in anaerobic reactors. COD removal efficiencies showed approximate results in every two reactors. However, the COD removal efficiency in ABR is higher 2-4% compared to AMBR. pH values in effluent of every two anaerobic reactors were between optimum pH values (6.5 and 8.3) as reported by Speece, (1996). TVFA concentrantrations were below 350 mg/l in AMBR reactor and below 450 mg/l throughout increasing NB loading rate and HRT study. Stable reactor performance is indicated by an effluent TVFA concentration below 500 mg/l (Willetts, 1999). This shows that the reactor operation was stable since the effluent VFA was <500 mg/l in every two anaerobic reactor through continuous operation. Furthermore, TVFA/Bic.Alk. ratios in the effluent of AMBR and ABR reactor were below 0.4 indicating the stable of AMBR and ABR reactors as reported by Behling et al., (1997).

Total and methane gas productions in AMBR and ABR reactor showed respect results throughout NB loading rates. Total gas productions varied between 1.8 and 2.8 l/day in AMBR and between 1.9 and 2.4 l/day in ABR, methane gas production also varied between 0.7 and 1.3 l/day in AMBR reactor and between 0.8 and 1.7 l/day in ABR reactor with increasing NB loading rates. ABR reactor showed a good resistance to high NB loadng rates compared to AMBR reactor. AMBR reactor deteriored after a NB loading rates of 9.63 g/m^{3}day. However ABR reactor showed a good performance even as high as a NB loading rates as high as 67.4 g/m^3.day. Total and methane gas productions in ABR reactor were higher than those in AMBR at HRTs varied from 10.38 days to 1 day. This shows the performance of ABR at high organic loading rates (lower HRTs).

In the treatment of wastewaters containing NB in compartmentalised reactors such as AMBR and ABR can be used, effectively. It is believed that these compartments would contribute to the further reduction in COD and NB.

Table 4.17 Comparison of AMBR and ABR reactors treating NB.

	AMBR reactor		**ABR reactor**	
	NB loading rate (g/m^3 day)	**HRT (days)**	**NB loading rate (g/m^3 day)**	**HRT (days)**
	0.96- 9.63	**1 - 10.38**	**1.9- 67.4**	**1 day -10.38**
COD removal efficiency (%)	85% -94%	80% -92%	80% -92%	84% -94%
NB removal efficiency (%)	100%	100%	100%	100%
The effluent pH	7.5-8.1	7.3-7.5	7.3-7.6	7.2-7.5
The effluent TVFA (mg/l)	0-30	0-350	0-32	0-480
The effluent Bic.Alk. (mg/l)	2890-3475	2100-3170	3000-3375	2060-3079
The effluent TVFA/Bic.Alk.	0-0.01	0.-0..2	0-0.02	0-0.19
Total gas (l /day)	1.8-2.8	2.8-11.8	1.9-3.4	2.8-17.6
Methane gas (l/day)	0.7-1.3	1.3-3.3	0.8-1.7	1.2-5.2
Methane percentage (%)	42-48	28-47	38-50	29-43

4.2.5 Determination of Kinetic Constants

Determination of kinetic constant of an AMBR and an ABR reactor is a useful tool to be able to describe and predict the performance of the anaerobic system. Recent literature surveys showed that the kinetic of AMBR and ABR reactors treating wastewater containing p-NP has not been investigated. Therefore in this study, the kinetic constant of the AMBR and ABR treating p-NP were evaluated according to the experimental data at six HRTs. In order to determine the most suitable biokinetic model in the AMBR and ABR treating p-NP, some kinetic models such as Monod, Grau first order, Grau second-order, Modified Stover-Kincannon, Zero, First and Second order kinetic models were applied to the experimental results obtained from the continuous operation. The interpretions of the models and the kinetic constants were performed in this step. Furthermore, gas production kinetics in AMBR and ABR reactors were determined using Modified Stover-Kincannon, Van der meer -Heertjes and Chen-Hasminoto models.

4.2.5.1 Kinetics of p-NP and COD Removals in AMBR

In order to obtain the kinetic coefficient for different kinetic models the AMBR reactor was operated with synthetic wastewater containing p-NP at six different HRTs through 185 days of operation period**.** The results obtained during the continuous operation of the AMBR at six different HRTs under steady state conditions are summarized in Table 4.18.

Table 4.18 Experimental data obtained under steady state conditions in AMBR reactor at six different HRTs.

HRT (days)	**10.38**	**5.19**	**3.4**	**2.4**	**1.5**	**1**
Operation period (day)	0-35	36-69	70-97	98-136	137-169	170-185
Sludge retention time (day)	340	280	180	151	125	110
Effluent COD con (mg/l)	266	230	294	345	421	570
COD removal eff (%)	92	92	90	89	86	82
Effluent p-NP con. (mg/l)	2.05	2.3	3.15	3.4	4.45	5.75
p-NP removal eff (%)	95	94	92	92	89	86
Total gas production rate (ml /day)	2160	3420	5280	8880	9600	12240
Methane gas production rate (ml /day)	980	1560	2340	3360	3554	3780
Methane percentage (%)	47	45	44	38	37	31
pH	7.3	7.3	7.3	7.1	7.3	7.3
TVFA (mg/l)	23	60	100	128	147	177
Bic.Alk. (mg/l)	3316	3286	3256	3381	3160	3125
TVFA/Bic.Alk.	0.01	0.02	0.03	0.04	0.05	0.06
Y (methane yield) m^{3}CH$_4$/g COD removed	0.26	0.21	0.2	0.21	0.14	0.11

4.2.5.1.1 Monod Kinetic Model. Six steady state sets datas were used to determine the kinetic constants for Monod Model. Figure 4.81 was plotted from the Eq 3.20 (See chapter 3.6.1.1.1) for determining the values of Y and k_d in this model. Growth yield coefficient (Y) (gVSS/gCOD) and endogenous decay coefficient (k_d) (day^{-1}) values calculated from the intercept and the slope of the straight line illustrated in Figure 4.81 (a) and (b) as 0.96 g VSS /g COD and 0.01 day^{-1}, respectively, with regression coefficient of R^2= 0.56, (y= 1.041x+ 0.0105) for COD. Y and k_d values was calculated as 58.83 g VSS/g p-NP and 0.012 day^{-1}, respectively, with regression coefficient of R^2= 0.75, (y= 0.017x+ 0.0002) for p-NP. The values of maximum specific substrate utilization rate (μ_{max}) (mgCOD/mgSS.day) and half saturation concentration (K_S) (mg/l) for COD and p-NP were determined from Figure 4.82 (a) and (b) using Eq (3.15). (μ_{max}) and (K_S) for COD were calculated as 0.048 1/day and 0.39 mg/l, respectively with regression coefficient of R^2= 0.25, (y= 8.15x+ 20.7), and the same parameters for p-NP were calculated as 0.045 1/day and 1.28 mg/l, respectively with regression coefficient of R^2= 0.88, (y= 0.209x-3.04). The effluent COD and p-NP concentrations can be calculated as follows from the Equation 3.20. Predicted and observed the effluent COD and p-NP concentrations were gived in Table 4.19.

$$S_e = S_i - \left[\left(\frac{q_h * X_r}{Y}\right) * \left(\frac{1}{q_c} + k_d\right)\right] \tag{4.1}$$

$$N_e = N_i - \left[\left(\frac{q_h * X_r}{Y}\right) * \left(\frac{1}{q_c} + k_d\right)\right] \tag{4.2}$$

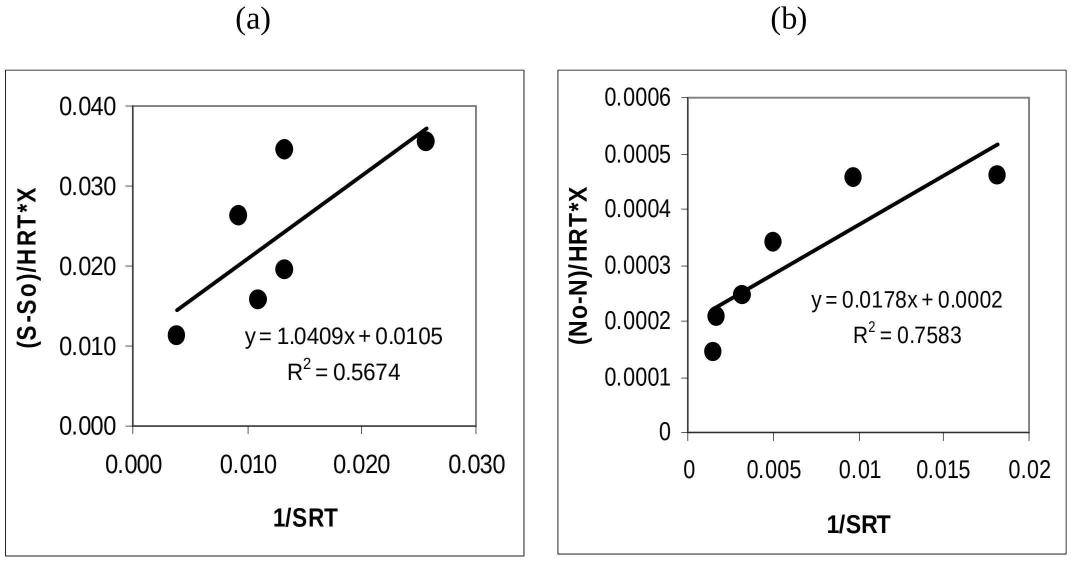


Figure 4.81 Determination of yield coefficient (Y) and death rate constant (k_d) values for COD (a) and p-NP (b).

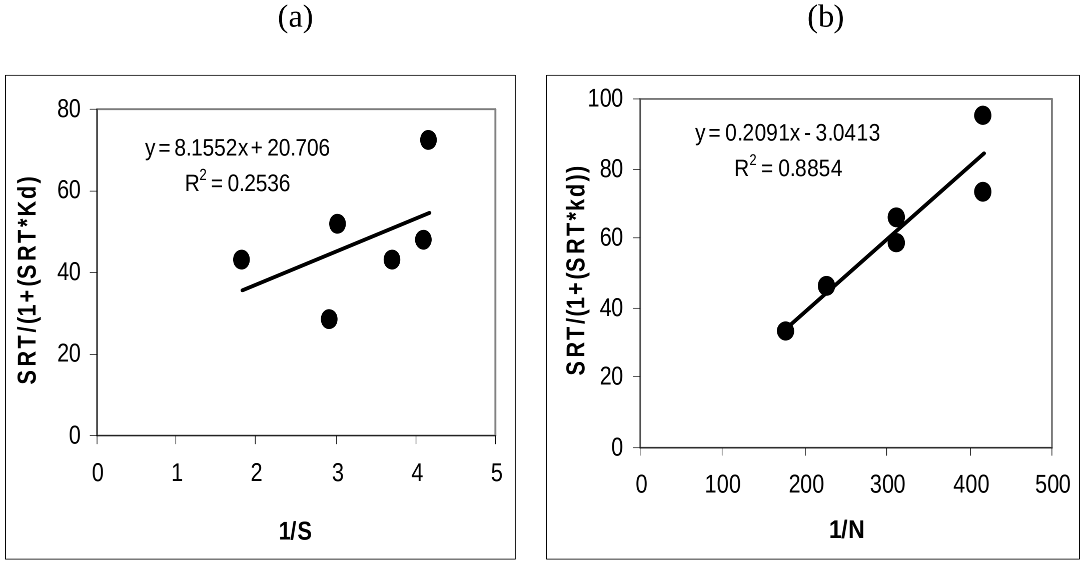


Figure 4.82 Determination of maximum specific substrate utilization rate (μ_{max}) and half saturation concentration (K_S) values for COD (a) and p-NP (b).

Table 4.19 Predicted and observed effluent COD and p-NP concentrations in Monod kinetic model.

Monod kinetic model				
HRT (day)	Observed effluent COD con. (mg/l)	Observed effluent p-NP con. (mg/l)	Predicted effluent COD con. (mg/l)	Predicted effluent p-NP con.(mg/l)
10.38	240	2.4	14	14
5.19	243	2.4	22	14
3.4	269	3.2	25	15
2.4	330	3.2	20	17
1.5	343	4.4	39	22

4.2.5.1.2 Grau First Order Substrate Removal Kinetic. In order to determine p (k_f=p*S_i) (l/day) and first order multicomponent substrate removal costant (k_f')(l/day) the kinetic constants, equation 3.26 was plotted in Figure 4.83. The values of p calculated from the slope of the line given on the graph. k_f was equal to k_f'*X (mg/l.day). The values of p, k_f and k_f' were found as 0.066 1/day, 0.207 g/l.day and 0.004 1/day for COD with regression coefficient R^2= 0.53, (y= 0.0657x+ 2.04). The aforementioned kinetic constants were found as 0.077 1/day, 3.08 g/l.day and 0.06 1/day for p-NP with regression coefficient R^2= 0.63 (y=0.077x+2.16). The effluent substrate concentration depend on k_f' and microorganism comcentration in the reactor (X). If k_f value is low, the effluent substrate concentration will be higher, while the removal efficiency will be lower. The effluent COD and p-NP concentrations can be calculated as follows from the Equation 3.25. In order to test the validity of the models the results obtained from the experimental analysis (observed values) were compared with the values obtained from the models (predicted values). The observed and predicted values for the effluent COD and p-NP concentrations are shown in Table 4.20.

$$e^{Se} = e^{Si} - \left(\frac{k_f '* q_h * X_r '}{S_i} \right) \tag{4.3}$$

$$e^{Ne} = e^{Ni} - \left(\frac{k_f{}' * q_h * X_r{}'}{N_i} \right) \tag{4.4}$$

(a) (b)

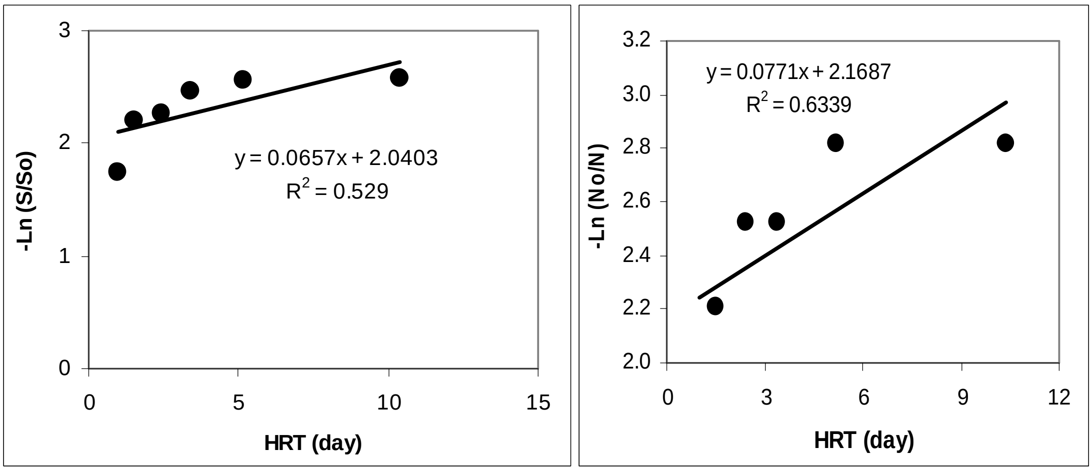


Figure 4.83 Determination of kinetic constants (p, kf and kf ’) for COD (a) and p-NP (b).

4.2.5.1.3 Grau Second-Order Multicomponent Substrate Removal Model. In order to determine a (Si/k_S*X_r) (day^{-1}), b (dimensionless) and second order substrate removal rate constant (k_S) (day^{-1}) kinetic constants for COD and p-NP, Equation 3.30 were plotted in Figure 4.84 (a) and (b). The values of a and b were calculated from the intercept and slope of the straight line on graph. The values of a, and b were found to be 0.096 day and 1.071 (dimensionless) with a regression coefficient of R^2= 0.87, (y= 0.855x+ 0.993) for COD (a). Second order mticomponent substrate removal rate constant (k_S) was calculated as 0.654 1/day from the equation a= So/(k_S.X), indicating the substrate removal for each unit of microorganism depends on second order substrate removal rate constant (k_S). The values of a, b and k_S were found as 0.0967 day, 1.0967 (dimensionless) and 0.0082 day^{-1} with regression coefficient R^2= 0.89, (y=0.636x+1.035) for p-NP (b). The effluent substrate concentration or substrate removal efficiency is related with influent substrate concentration and Grau second order kinetic constant as described in Equation 3.30. The effluent COD and p-NP concentrations can be predicted from Eqs.4.5 and 4.6.

$$S_e = S_i(1 - \frac{1}{(1.01 + 0.096/q_h)} \tag{4.5}$$

$$N_e = N_i(1 - \frac{1}{(1.09 + 0.0096/q_h)} \tag{4.6}$$

In order to test the validity of the models the results obtained from the experimental analysis (observed values) were compared with the values obtained from the models (predicted values). The observed and predicted values for the effluent COD and p-NP concentrations are shown in Table 4.20. As can be seen from this table, the effluent COD and p-NP values predicted in Grau Second Order kinetic model were close to the experimental results in AMBR.

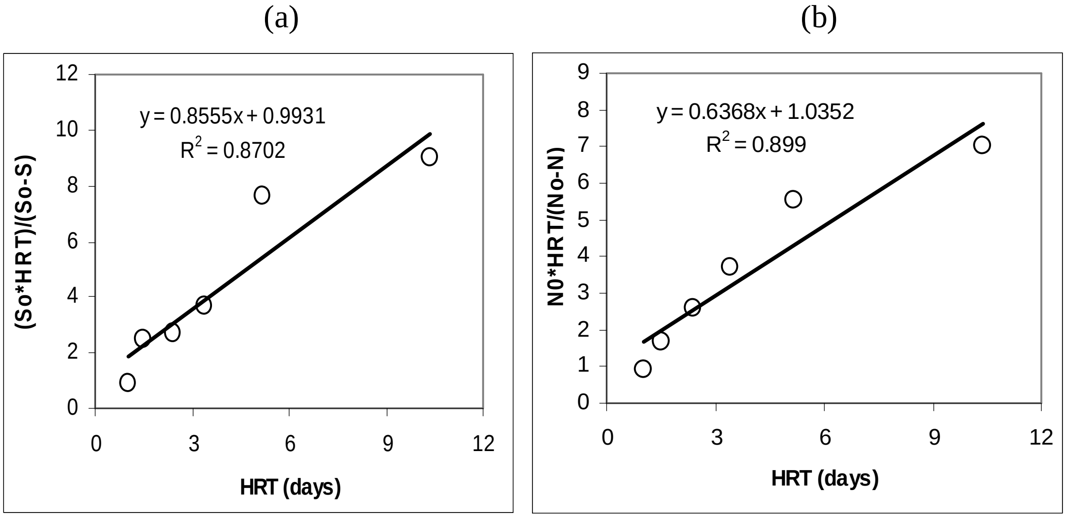


Figure 4.84 Determination of kinetic constants (a, b and k_s) for COD (a) and p-NP (b) for Grau second order multicomponent substrate removal model.

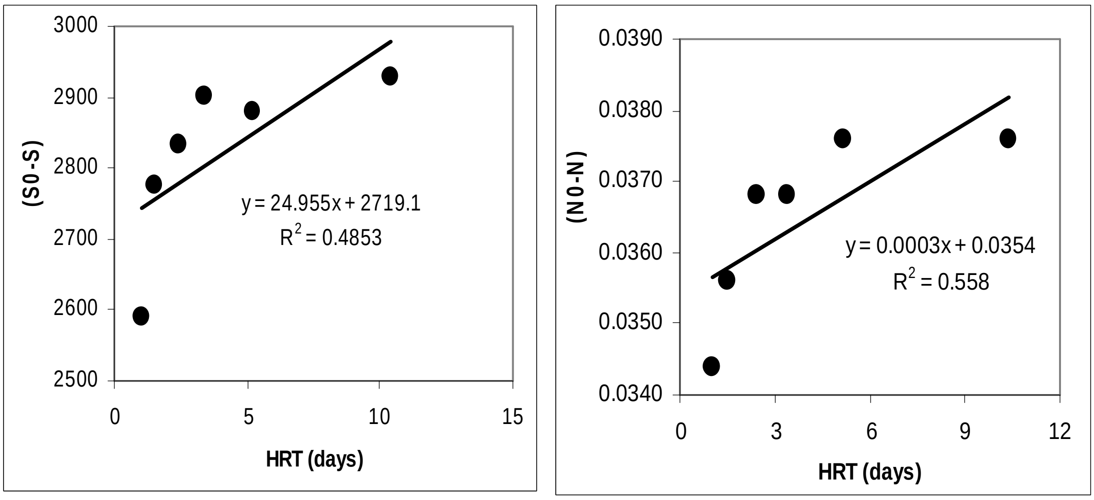
Table 4.20 Predicted and observed effluent COD and p-NP concentrations in Grau second order multicomponent substrate removal model.

Grau second order multicomponent substrate removal model				
HRT (day)	Observed effluent COD con.(mg/l)	Observed effluent p-NP con. (mg/l)	Predicted effluent COD con. (mg/l)	Predicted effluent p-NP con. (mg/l)
10.38	240	2.4	210	2.9
5.19	243	2.4	256	4.14
3.4	269	3.2	286	4.45
2.4	330	3.2	320	4.82
1.5	384	4.4	389	6.1

4.2.5.1.4 Zero Order Substrate Removal Model. The value of zero order kinetic costant (k_0) was obtained from the slope of the line by plotting S_0-S versus θ_h in Eq. (3.34). Figure 4.85 (a) and (b) shows the plot between S_0-S and θ_h and N_0-N and θ_h, respectively. k_0 was calculated as 24.95 mg/l.day with regression coefficient of 0.485 (y=24.95+2719.1) for COD. k_0 was calculated as 0.0003 mg/l.day with regression coefficient of 0.558 (y=0.0003+0.0354) for p-NP. The effluent COD and p-NP concentrations calculated from Eq. (3.34) depending to HRT (θ_c) and k_0. If k_0 value is low, the effluent substrate concentration will be high (removal efficiency is low). The effluent substrate concentrations will increase when HRT decreased. The actual and predicted values for the effluent COD and p-NP concentrations are calculated from Egs (4.7) and (4.8). Table 4.21 shows the effluent COD and p-NP values predicted from the in Grau second order model was not show a good agreement with the results obtained from the experimental studies.

$$Se = S_i - (0.485 * q_h) \tag{4.7}$$

$$Ne = N_i - (0.558 * q_h) \tag{4.8}$$



(a) (b)

Figure 4.85 The model plot of zero order substrate removal for COD (a) p-NP (b).

Table 4.21 Predicted and observed effluent COD and p-NP concentrations in Zero order Substrate removal model.

Zero order Substrate removal model				
HRT (day)	Observed effluent COD con.(mg/l)	Observed effluent p-NP con.(mg/l)	Predicted effluent COD con.(mg/l)	Predicted effluent p-NP con.(mg/l)
10.38	240	2.4	2911	40
5.19	243	2.4	2994	40
3.4	269	3.2	3085	40
2.4	330	3.2	3104	40
1.5	384	4.4	3081	40

4.2.5.1.5 First Order Substrate Removal Model. First order kinetic constant (k_1) was obtained from the slope of the line by plotting S_0-S/θ_c versus S in Eq (3.36). Figure 4.86 (a) and (b) shows the plot between (S_0- S)/HRT and S and (N_0- S)/θ_h and N_i. k_1 was calculated as 6.978 1/day with correlation coefficient of 0.88, (y=6.967x-1.078) for COD. Similarly k_1 for p-NP was calculated as 0.009 day^{-1} with regression coefficient of 0.97, (y=0.009x-0.0161). The effluent COD and p-NP concentrations were calculated as follows using Eq. 3.37. The effluent substrate concentrations depend on k_1 and HRT. The high k_1 values results with high substrate removal efficiencies. The actual and predicted values for the effluent COD and p-NP concentrations are showed in Table 4.22. As can be seen from this table, the effluent COD and p-NP values predicted from the first order kinetic model was not close to the results obtained from the experimental studies.

$$Se = \frac{S_i}{6.978 * q_h + 1} \tag{4.8}$$

$$Ne = \frac{N_i}{0.009 * q_h + 1} \tag{4.9}$$

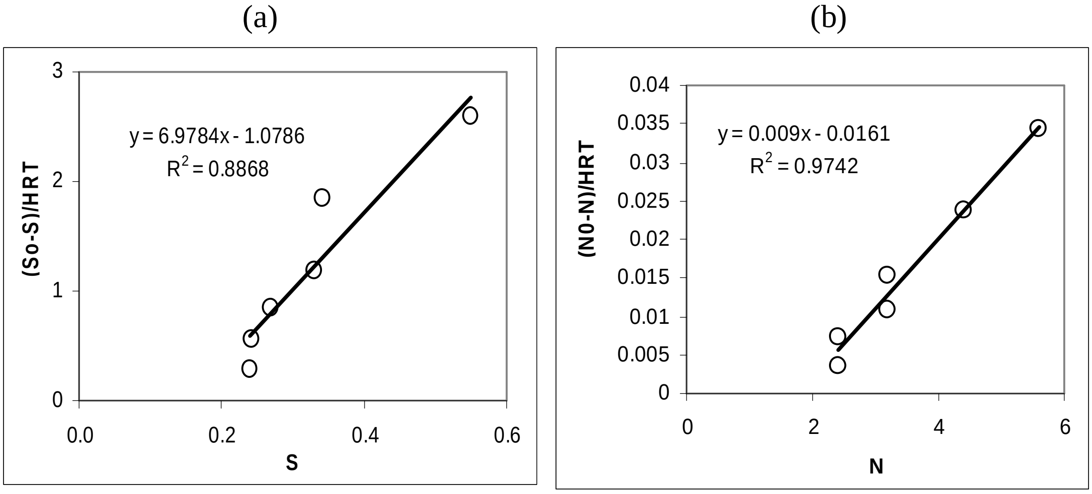


Figure 4.87 Detemination of first order substrate removal for COD (a) and p-NP (b).

Table 4.22 Predicted and observed effluent COD and p-NP concentrations in first order model.

First order substrate removal model				
HRT (day)	Observed effluent COD con.(mg/l)	Observed effluent p-NP con.(mg/l)	Predicted effluent COD con.(mg/l)	Predicted effluent p-NP con. (mg/l)
10.38	240	2.4	43	37
5.19	243	2.4	84	38
3.4	269	3.2	128	39
2.4	330	3.2	178	39
1.5	384	4.4	272	39

4.2.5.1.6 Second Order Substrate Removal Model. In the second oreder substrate removal kinetic model (k_2) was obtained from the slope of the line by plotting S_i-S_e/θ_h versus S_i^2 in Eq (3.39). Figure 4.87 (a) and (b) shows the plot between (S_0- S)/ θ_h and S^2 and (N_0- N)/θ_h and N^2. k_2 for COD was calculated as 0.0008 l/mg.day with regration coefficient of 0.82 (y=0.008x+247.2). Similarly k_2 for p-NP was calculated as 0.001 day^{-1} with correlation regration of 0.95 (y=0.0011x+0.0003). The effluent COD and p-NP concentrations were calculated as follows using Eq. 3.39. The actual and predicted values for the effluent COD and p-NP concentrations are showed in Table 4.23. The effluent COD and p-NP values predicted from the in first order removal model was higher than the results obtained from the experimental studies.

$$\frac{S_i - S_e}{q_h} = 0.0008 * S_e^{\,2} \tag{4.10}$$

$$\frac{N_i - N_e}{q_h} = 0.001 * N_e^{\,2} \tag{4.11}$$

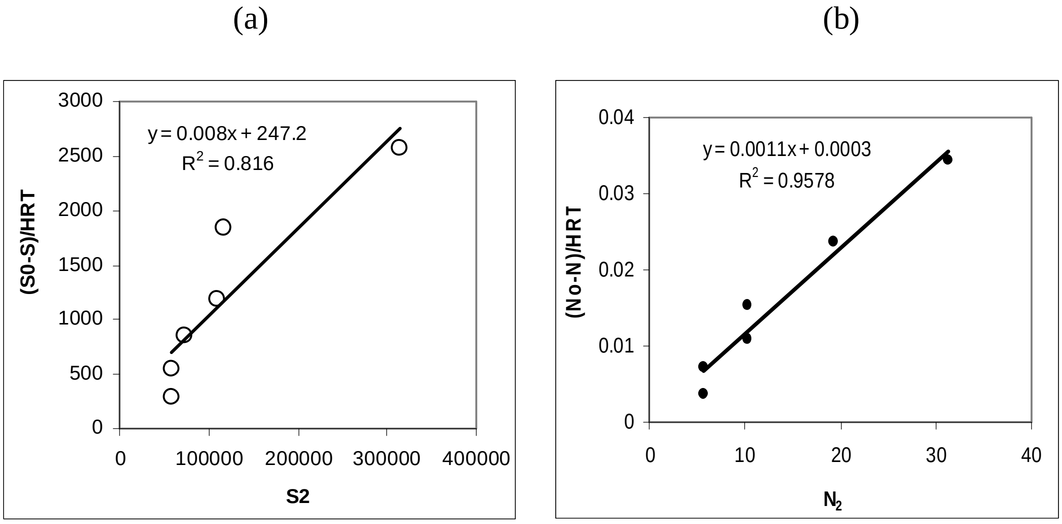


Figure 4.87 Determine of second order substrate removal for COD (a) p-NP (b).

Table 4.23 Predicted and observed effluent COD and p-NP concentrations in second order model.

Second order substrate removal model				
HRT (day)	Observed effluent COD con.(mg/l)	Observed effluent p-NP con.(mg/l)	Predicted effluent COD con.(mg/l)	Predicted effluent p-NP con.(mg/l)
10.38	240	2.4	561	30
5.19	243	2.4	755	34
3.4	269	3.2	912	35
2.4	330	3.2	1050	36
1.5	384	4.4	1248	38

4.2.5.1.7 Modified Stover-Kincannon Model. Figure 4.88 shows the graph plotted between reciprocal of total removed organic loading removal rate, $[V/(Q^*(S_i\text{-}S_e)]$, against to the reciprocal of total organic loading rate, $V/(Q^*S_i)$ using Eq (3.42). Since the plot of $[V/(Q^*(S_i\text{-}S_e)]$ versus $V/(Q^*S_i)$ was found to be linear, linear regressions (least squares method) were used to determine the intercept ($1/R_{max}$) and the slope (K_B/R_{max}). Saturation value constant (K_B) and maximum utilization rate (R_{max}) for COD and p-NP were calculated from the line plotted on graph given in Figure 4.85 (a) and (b). K_B and R_{max} was found as 29.498 gCOD /l.day and 33.55

gCOD/l.day, respectively with high regression coefficient ($R^2=1$; y= 1.0696x+0.0339) for COD. Similarly, the R_{max} and K_B values for p-NP were obtained as 0.407 g p-NP/l.day and 0.428 g p-NP/l.day, respectively, with high regression coefficient ($R^2=1$, y= 1.0528x+2.4551). Stover Kincannon model suggest that the substrate removal rates (COD and p-NP) are affected by the organic loading rate entering to the reactor as described in Eq (3.42). The effluent COD and p-NP concentration can be calculated from Eq (4.12) and (4.13) using Eqs (3.44) and (3.45).

$$Se = S_i - \frac{33.11 * S_i}{30.96 + (S_i / q_h)} \tag{4.12}$$

$$N_e = N_i - \frac{0.407 * N_i}{0.428 + (N_i / q_h)} \tag{4.13}$$

The results obtained from the experimental analysis (observed values) were compared with the values obtained from the models (predicted values). The actual and predicted values for the effluent COD and p-NP concentrations are showed in Table 4.24. As can be seen from this table, the effluent COD and p-NP values predicted in Stover Kincannon model was show a good agreement with the results obtained from the experimental studies. In other word, the predicted values were very close to the experimental results when Stover Kincannon model was applied to the AMBR.

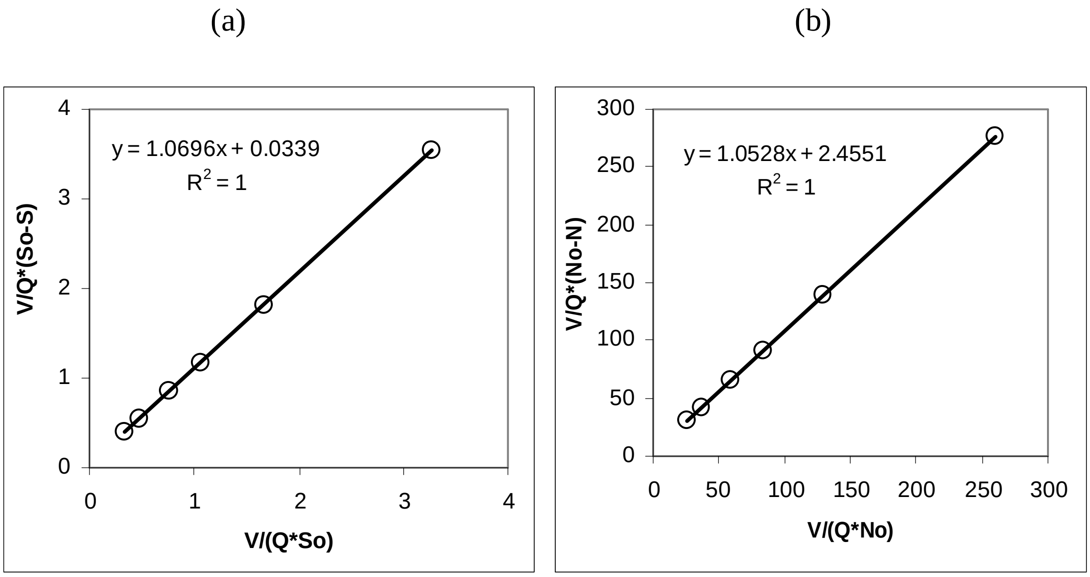


Figure 4.88 Determination of kinetic constants (R_{max} and K_B) in Stover-Kincannon model for COD (a) and p-NP (b).

Table 4.24 Predicted and observed effluent COD and p-NP concentrations in Modified Stover-Kincannon model.

Modified Stover-Kincannon model				
HRT (day)	Observed effluent COD concentration (mg/l)	Observed effluent p-NP concentration (mg/l)	Predicted effluent COD concentration (mg/l)	Predicted effluent p-NP concentration (mg/l)
10.38	240	2.4	210	2.9
5.19	243	2.4	256	4.14
3.4	269	3.2	286	4.45
2.4	330	3.2	320	4.82
1.5	384	4.4	389	6.1

4.2.5.1.8 Evaluation of the Kinetic Models for AMBR Reactor. All kinetic coefficients calculated from the models are summarized in Table 4.25 with regration coefficients. The kinetic data showed that Stover-Kincannon and Grau second order multicomponent substrate removal kinetics were more appropriate models than other models for predicting the performance of the lab scale AMBR reactor when the regression coefficients and kinetic coefficients were compared with each other.

The yield coefficiency (Y) was higher compared to the death rate coefficient (k_d) in Monod kinetic model. Half saturation constant (K_S) was lower compared to initial COD concentration of 3000 mg/l. Maximum specific grow rate (μ_{max}) was higher compared to the death rate constant (k_d). This can be explained by the long HRT and sludge retention time. High K_S value indicates a higher affinity to COD treating anaerobic methanogens. K_S values estimated from the Monod model is very lower (K_s= 0.39 mg/l) than acceptable values. In the study performed by Pavlostathis & Giraldo-Gomez, (1991) μ_{max}, Y, and K_S values were 0.77–6.67 mg COD (mgVSS/day), 0.04–0.11 mg VSS/mgCOD, and 105–3180 mg COD/l, respectively, for anaerobic oxidation of long-chain fatty acids. In order to test the validity of the Monod model the results obtained from the experimental analysis (observed values) were compared with the values obtained from the models (predicted values). As can be seen from Table 4.19 the predicted values were lower than observed effluent COD values. This could be explained by lower Ks value.

Maximum substrate removal rate (R_{max}=33.55 gCOD/l day) in the Modified Stover-Kincannon model was higher than the Zero order (k_0=24.95 mgCOD/l day) and Grau first order (k_f=0.2 gCOD/l day) kinetic models. High COD utilization rate increase the reactor efficiency. In order to test the validity of the Zero, First and Second order kinetic models the results obtained from the experimental analysis were compared with the values obtained from the models (predicted values). As a result, it was not observed a good agreement with the results obtained from the experimental studies and predicted values (See Table 4.21, 4.22, 4.23).

The regression coefficients for COD and p-NP under six different HRTs were higher in Stover Kincannon kinetic model (R^2=1) compared to Grau second order model (R^2=0.89). Furthermore the kinetic constants determined in Stover Kincannon model are more meaningful than that observed in Grau second order kinetic model. The maximum substrate utilization rate (R_{max}) is higher and the saturation value constant (K_B) is lower during COD degradation. High COD utilization rate (R_{max}) increase the reactor efficiency while low substrate saturation constant (K_B) indicates the utilization of COD and p-NP by the methanogens in the AMBR. R_{max} value obtained from the study performed by Karim & Gupta (2006) was 15.5 mg/l day in an upflow anaerobic sludge blanket reactor treating 30 mg/l of p-NP at a p-NP loading rate of 180 mg p-NP/l.day. This result is significantly lower than the R_{max} value obtained from this study. Low saturation values (K_B) showed that there is no any accumulation of COD and p-NP in the anaerobic reactor resulting in high affinity of substrate to the anaerobic methanogenic bacteria.

a, b and k_s kinetic constants calculated from the Grau second order kinetic model showed that a kinetic constant depends to influent COD, p-NP concentrations and it was influenced by the inverse of second order substrate removal rate constant and microorganism concentrations. The a kinetic constant will be increased with initial substrate concentration while will be decreased as the second order substrate removal rate and microorganism concentration increased. The maximum substrate removal rate constant (k_S) will be increased as the COD removal efficiencies increased, depending to initial substrate (S_i) and microorganism concentration (X_r) in the reactor.

In order to test the validity of the Grau second order kinetic model and Stover Kincannon kinetic model the results obtained from the experimental analysis (observed values) were compared with the values obtained from the models (predicted values). As can be seen from Table 4.24, the predicted values from Stover Kincannon model were very close to the experimental results. The effluent COD and p-NP values predicted in Grau second order model was not show a good agreement with the results obtained from the experimental studies as compared to Stover Kincannon model (See Table 4.20).

Table 4.26 summarizes the constants determined from the Monod, Stover-Kincannon and Grau second order multicomponent substrate removal kinetics from different wastewater and reactors. K_S and μ_{max} values (1.24 mg/l and 0.037 day^{-1}, respectively) obtained in this study for Monod model was lower than then reported by Işık & Sponza (2005) (4000 mg/l, 0.105 day^{-1}, respectively **)** in UASB reactor treating simulated textile wastewater and by Sponza & Uluköy (2007) (560 mg/l, 0.213 day^{-1}, respectively) in UASB reactor treating 2,4 DCP.

In this study, the saturation constant (K_B) (29.49 g/l.day) and maximum utilization rate (R_{max}) (33.55 g/l day) values obtained from the Modified Stover-Kincannon model are higher than those obtained by Işık & Sponza (2005) (7.5 g/l.day and 8.2 g/l.day, respectively) in UASB reactor treating simulated textile wastewater and than those obtained by Kapdan, (2005) (12.9 g/l.day and 37.7 g/l.day, respectively) in packed colum treating textile dye stuff in modified Stover-Kincannon model. High COD utilization rate (R_{max}) increase the reactor efficiency while low substrate saturation constant (K_B) indicates the utilization of COD and p-NP by the methanogens in the AMBR. The multicomponent substrate rate constant (k_S) value obtained from the Grau second order model in this study was higher than those obtained by Işık & Sponza (2005) in a UASB reactor (k_S=0.337 day^{-1}). Furthermore, the k_S value was 0.217 day^{-1} in a study performed by Ubay, (1989) in a UASB reactor treating municipal wastewater using grau second order kinetic model.

A good linear relationship was obtained between observed and predicted effluent COD and p-NP concentrations under six different HRTs in Modified Stover Kincannon Kinetic model (y= 1.309x-98.69 and R^2=0.96, and y=1.188x-0.5463 and R^2=0.97, respectively, for COD and p-NP) (See Figures 4.89 and 4.90). The linear relationship was obtained observed and predicted effluent COD and p-NP concentrations under six different HRTs in Grau second order kinetic (y=1.3215x-79.432, R^2=0.83; y=0.887x-0.7212 and, R^2=0.83, respectively, for COD and p-NP) (See Figures 4.91 and 4.92).

The linear relationship between observed and predicted effluent COD and p-NP concentrations in Grau second order kinetic is lower than that obtained from the

Modified Stover- Kincannon model. The HRT versus observed and predicted effluent COD and p-NP concentrations clearly showed that the predicted effluent COD and p-NP concentrations are closer to the observed values in Stover-Kincannon Kinetic model (See Table 4.24).

Table 4.25 Kinetic parameters of AMBR reactor treating p-NP.

		COD removal		**p-NP removal**	
Kinetic models	**Kinetic parameters**	**Values**	**Regression coefficients (R^2)**	**Values**	**Regression coefficients (R^2)**
Monod	Y (mgVSS/mgCOD)	0.96	0.56	58.83	0.25
	k_d (day^{-1})	0.01	056	0.012	0.25
	μ_{max} (day^{-1})	0.048	0.75	0.045	0.88
	k_{max} (μ_{max}/Y) (day^{-1})	0.05	0.75	0.0007	0.88
	K_s (mg/l)	0.39	0.75	1.28	0.88
Grau first order	p (l/day)	0.066	0.53	0.077	0.63
	k_f (g/l day)	0.207	0.53	3.08	0.63
	k_f'(l/day)	0.004	0.53	0.06	0.63
Grau second order	k_s (l/day)	0.654	0.87	0.0082	0.89
	a (day)	0.096	0.87	0.096	0.89
	b (dimensiones)	1.071	0.87	1.096	0.89
Modified Stover-Kincannon	K_B (g COD/l day)	29.49	1	0.428	1
	R_{max} (g COD/l day)	33.55	1	0.407	1
Zero order	k_0 (mg /l day)	24.95	0.48	0.0003	0.55
First order	k_1 (day^{-1})	6.978	0.88	0.009	0.97
Second order	k_2 (l /mg day)	0.0008	0.82	0.001	0.95

Table 4.26 Comparison of kinetic constants in the Grau second order, Modified Stover Kincannon and Monod models.

Model	**Substrate**	**Reactor type**	**Influent COD (mg/l)**	**HRT (day)**	**Kinetic parameters**				**Referance**
					k_s	**a**	**b**		
Grau second order	Synthetic (glucose)	ACR	14750	4.8-24	-	0.073	1.073		Anderson & Donnely, 1978
Grau second order	Brewery	AFBR	200-300	0.2-1.7	2.1	0.1	1.015		Anderson, Öztürk, & Saw 1990
Grau second order	Municipal wastewater	UASB	230–445	0.25–1.0	0.217	0.002	1.346		Ubay, 1994
Grau second order	Molasses	AHR	2000–15000	0.5–2	10.81	0.033	1.192		Büyükkamaci & Filibeli,2002
Grau second order	Simulated textile wastewater	UASB	4214	0.25–4.16	0.337	0.562	1.095		Işık & Sponza,2005
Grau second order	Synthetic (2,4-DCP)	UASB	3000	2-20 h	0.26	0.029	0.011		Sponza & Uluköy, 2007
Grau second order	Synthetic (p-NP)	AMBR	3000	1-10.38	0.654	0.096	1.071		This study
Grau second order	Synthetic (p-NP)	ABR	3000	1-10.38	0.231	0.032	0.97		This study
					R_{max}		***K_B***		
Stover Kincannon	Molasses	AHR	2000–15000	0.5–2	83.3		186.23		Büyükkamacı & Filibeli
Stover Kincannon	Soybean wastewater	AF	7520–11450	1–1.45	83.3		85.5		Yu, Wilson & Tay,1998
Stover Kincannon	Textile wastewater	UASB	4214	0.25–4.16	7.5		8.2		Işık & Sponza,2005
Stover Kincannon	Textile dye stuff	PC	OLR=2.5-8	-	12.99		37.69		Kapdan , 2005
Stover Kincannon	Synthetic (2,4-DCP)	UASB	3000	2-20 h	7.5		34.56		Sponza & Uluköy, 2007
Stover Kincannon	Synthetic (p-NP)	AMBR	3000	1-10.38	33.55		29.49		This study
Stover Kincannon	Synthetic (p-NP)	ABR	3000	1-10.38	9.94		9.7		This study
					μ_{max}	**Y**	**k_d**	**K_s**	
Monod	Simulated textile wastewater	UASB	4214	0.25–4.16	0.105	0.125	0.006	4000	Işık & Sponza, 2005
Monod	Synthetic (2,4-DCP)	UASB	3000	2-20 h	0.213	0.78	0.093	560	Sponza & Uluköy, 2007

UASB=upflow anaerobic sludge blanked reactor, AHR=anaerobic hybrid reactor, ACR=anaerobic contact reactor, AF=anaerobic filter, PC= Packed column, AMBR=anaerobic migrating blanked reactor, ABR=Anaerobic baffled reactor, **a and b=c**onstant, **k_s =** Grau second-order substrate removal rate constant (day^{-1}), **R_{max}** =maximum utilization rate (g COD/l day), **K_B** =saturation value constant (g COD/l day), **μ_{max}** =maximum specific growth rate (day^{-1}), ***Y***=growth yield coefficient (g VSS / g COD), **k_d** =endogenous decay coefficient (day^{-1}), **K_s =** half saturation concentration (mg/ l).

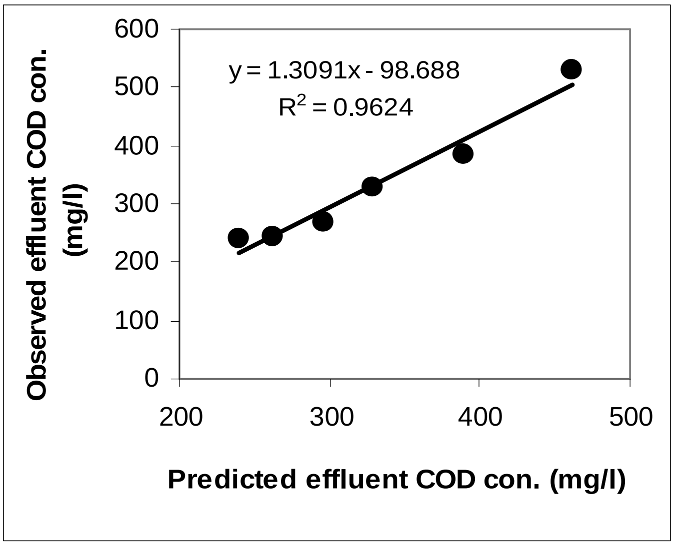


Figure 4.89 The observed effluent COD concentration and predicted effluent COD in Stover-Kincannon Kinetic model.

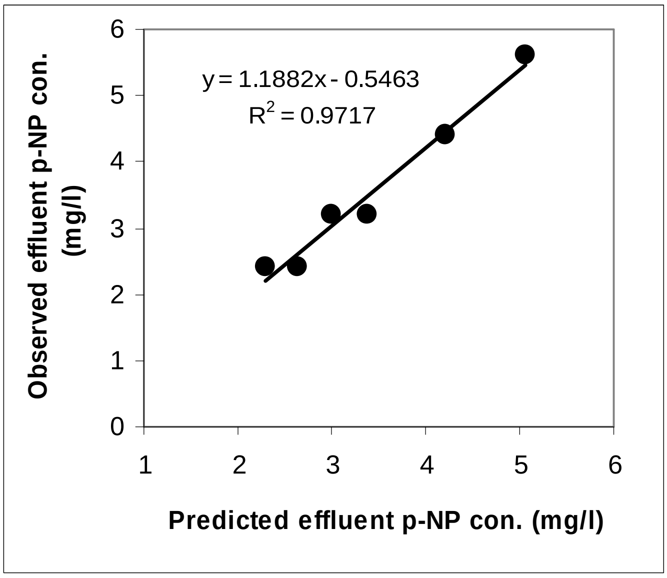


Figure 4.90 The observed effluent p-NP concentration and predicted effluent p-NP in Stover-Kincannon Kinetic model.

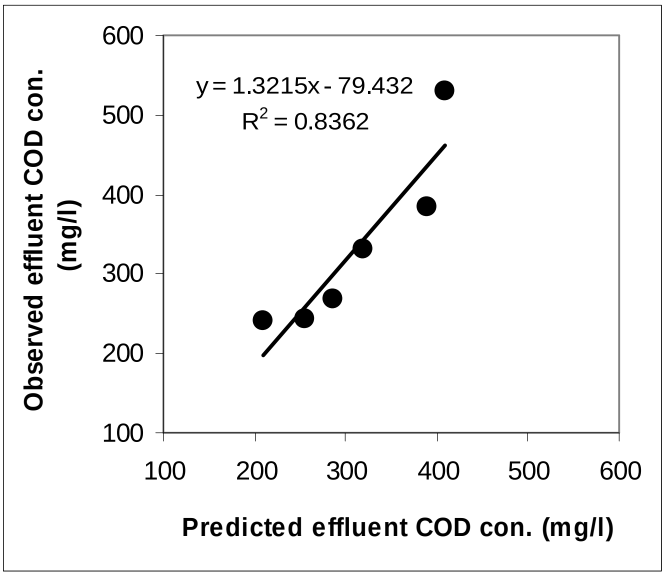


Figure 4.91 The observed effluent COD and predicted effluent COD concentrations in Grau second order kinetic model.

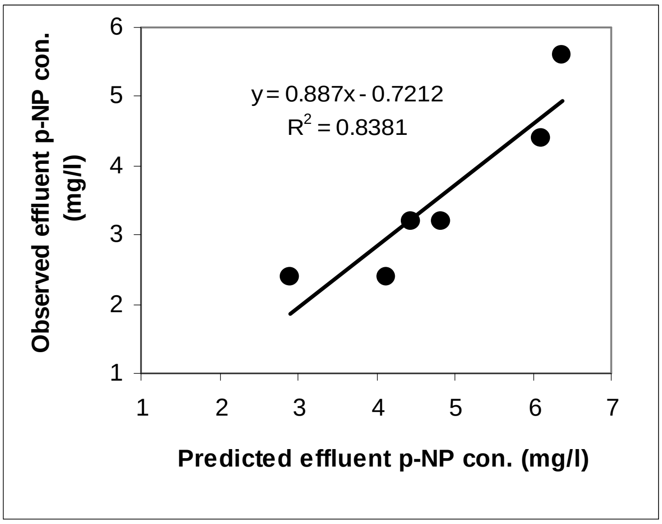


Figure 4.92 The observed effluent p-NP and predicted effluent p-NP concentrations in Grau second order kinetic model.

4.2.5.2 Biogas Production Kinetics in AMBR

Three biogas production kinetic models namely, *Modified Stover Kincannon, Van der Meer and Heertjes and Chen-Hasminoto* were evaluated for biogas production in AMBR reactor. The kinetic constants and regression coefficients of the models were compared in AMBR reactor.

4.2.5.2.1 Modified Stover-Kincannon Model. In order to determine the kinetic coefficients such as maximum specific gas production (G_{max}) and maximum methane gas production rate (M_{max}) Eqs (3.50) and (3.51) were used to calculate the total and methane gas productions in AMBR. The reciprocal of specific gas production (1/G) versus reciprocal of applied substrate loading rates (1/OLR) were plotted in order to determine the kinetic constants relevant to total and methane gas productions, respectively. The intercept and slope of the line result in $1/G_{max}$ and G_B/G_{max}, respectively. The maximum specific total gas production rate, G_{max}, and proportionality constant, G_B, were found to be 1666.7 ml/l.day and 2.83 (mg/l day), respectively (R^2 = 0.97; y= 0.0017x+0.0006) using Eq (3.50) (See Fig.4.93). The maximum methane gas production rate, M_{max}, and proportionality constant, M_B, were found to be 476.2 ml/l day and 1.67 mg/l day, respectively (R^2=0.98, y= 0.0035x+ 0.0021) using Eq (3.51) (See Fig. 4.94). In the Modified Stover Kincannon biogas kinetic model gas production rates (G_{max} and M_{max}) are related with the organic loading rates applied to the AMBR reactor and with proportionality constants as given in Equations (3.50) and (3.51). The production total gas and methane gas amounts can be calculated as Eq (4. 14) and (4.15).

$$\frac{1}{G}=\frac{2.83}{1667}\times\frac{1}{OLR}+\frac{1}{1667} \tag{4.14}$$

$$\frac{1}{M}=\frac{1.67}{476}\times\frac{1}{OLR}+\frac{1}{476} \tag{4.15}$$

G is specific total gas production rate (ml/l.day), while M is specific methane gas production rate (ml/l.day). OLR is organic loading rate (g/l.day).

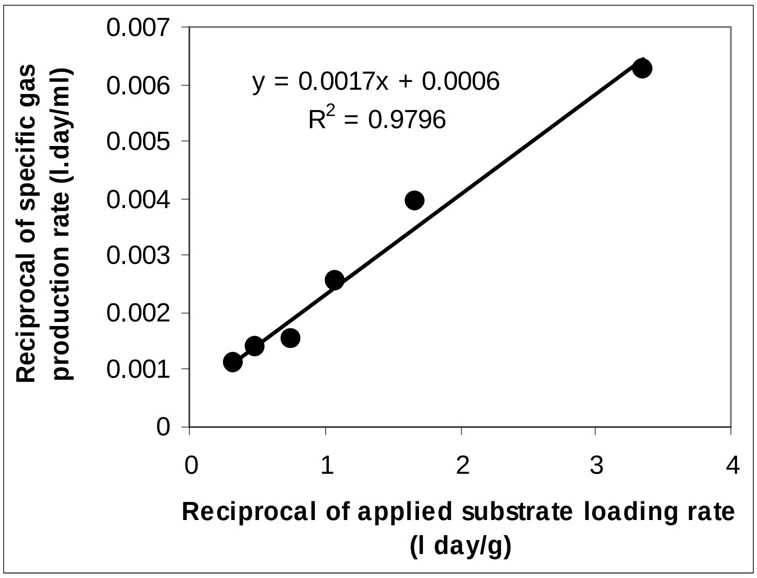


Figure 4.93 Determination of G_{max} and G_B in Modified Stover Kincannon model.

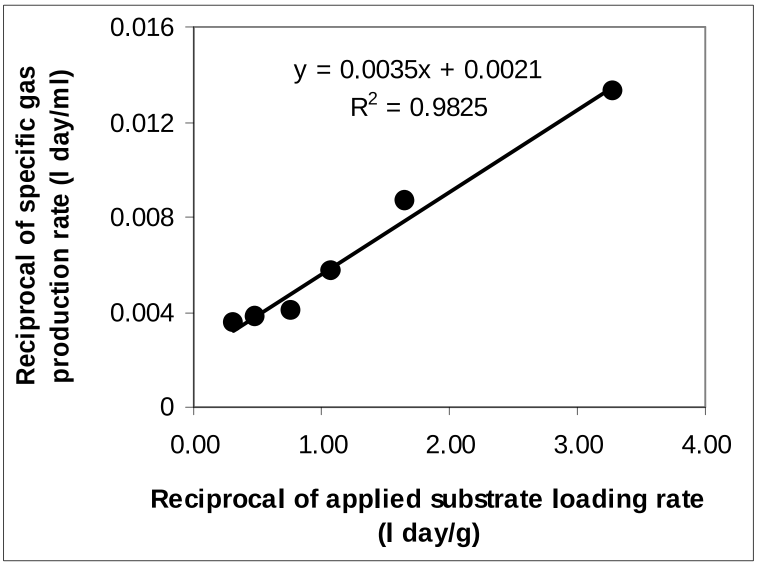


Figure 4.94 Determination of M_{max} and M_B in Modified Stover Kincannon model.

4.2.5.2.2 Van der Meer and Heertjes Model. Van der Meer and Heertjes kinetic constant (k_{sg}) was determined empirically from the slope of the line plotted between $Q(S_i\text{-}S_e)$ and CH_4 using Eq (3.52) (R^2=0.83,y= 0.0947x+1069.2)(See Figure 4.95). The kinetic constant of gas production (k_{sg}) was found as 0.0947 ml CH_4/mg COD removal. In this model the methane gas production is related with gas kinetic constant, flowrate applied to the AMBR reactor and removed substrate concentrations. The methane gas productions can be predicted using Eq (4.16). as follows:

$$V=0.0947\ Q\ (S_i\text{-}S_e) \tag{4.16}$$

The methane gas productions were found between 361 and 3164 ml/day at HRTs varied from 10.38 to 1 day according to Eq (4.16).

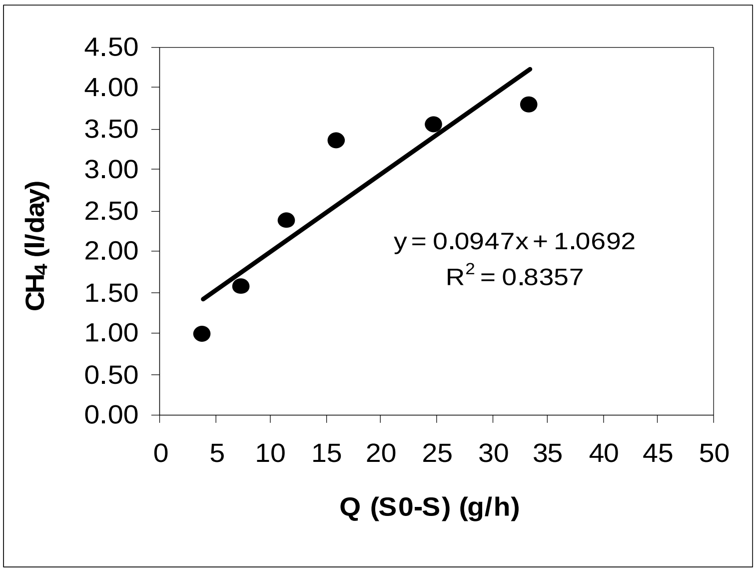


Figure 4.95 Determination of k_{sg} in Van der Meer and Heertjes model.

4.2.5.2.3 Chen and Hasminoto Model. The ultimate methane yield (B_{max}) was calculated as 0.0704 l CH_4 / g VS added (0.283 l CH_4/kg COD removed) from the intercept plotted between methane yield and 1/HRT using Eq. 3.56 (R^2=0.68, y= 3.531x-5.097) (See Figure 4.96). The maximum specific growth rate (μ_{max}) and k can be determined graphically by plotting the term (B_{max}/(B_{max}-B) versus θ_h. The slope of the straight line is equal to μ_{max}/k and the intercept is equal to (1-1/k). The maximum specific growth rate (μ_{max}) was found as 0.579 day^{-1} and Chen and Hasminoto kinetic constant (k) was found as 0.164 (dimensionless) (R^2=0.68, y= 3.531x-5.0974) (See Figure 4.97). In this model, ultimate methane yield and maximum specific growth rate of methanogens affected by hydraulic retention time and Chen and Hasminoto kinetic constant. The methane gas productions can be predicted as follows with re-aranged of Eq 3.56.

$$B = B_{\max} - \frac{B_{\max}.k}{q_h * m_{\max} + k - 1} \tag{4.17}$$

$$B = 0.0704 - \frac{0.0704 \times 0.164}{q_h * 0.579 + 0.164 - 1} \tag{4.18}$$

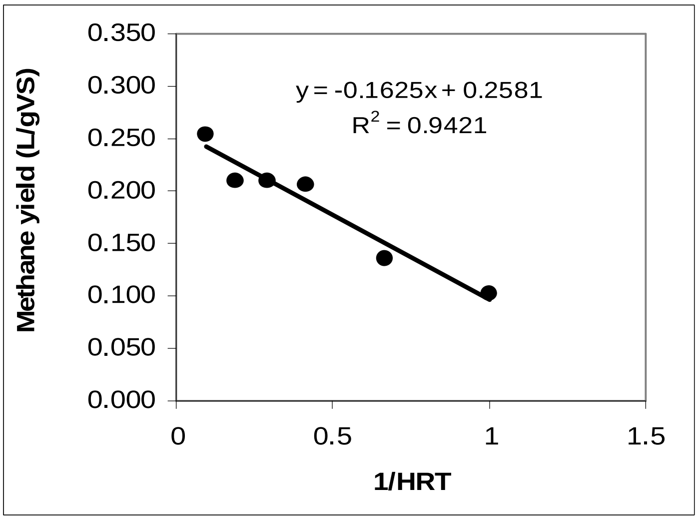


Figure 4.96 Determination of Y_{max} in Chen and Hasminoto model.

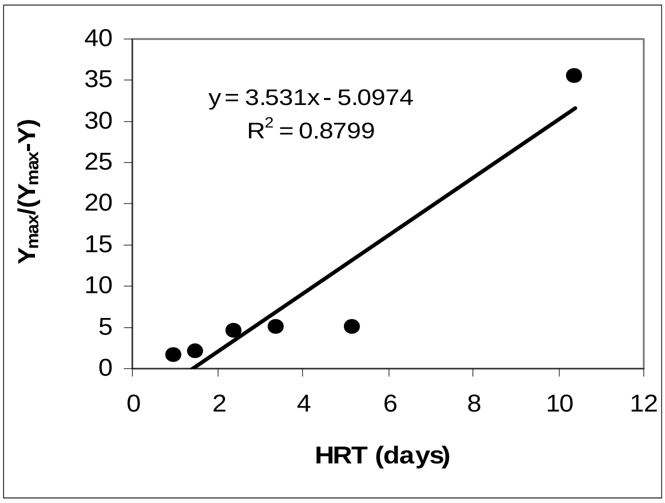


Figure 4.97 Determination of μ_{max} and k in Chen and Hasminoto model.

4.2.5.2.4 Evaluation of the Biogas Kinetic Models for AMBR Reactor. The methane and total gas productions calculated from the experimental studies were compared in three different kinetic models. The kinetic constants calculated from the models are summarized in Table 4.27. The results for observed and predicted total and methane gas productions are given in Table 4.28. The total and methane gas productions predicted from the Modified Stover-Kincannon model was very close to the experimental data compared to the other two models at six different HRTs. The linear relationships showed that the regression coefficient is higher in Modified Stover-Kincannon model compared to the other two models (See Table 4.27). Furthermore the kinetic constants relevant with gas production rates and the proportionality constants found from this model are meaningful for maximum anaerobic total and methane gas production rates (Speece, 1996).

The experimental methane gas production varied between 980 ml/day and 3800 ml/day. The predicted data from the Modified Stover-Kincannon kinetic model was close the experimental results (See Table 4.28). The maximum growth rate for methanogens (μ_{max}) in Chen and Hasminoto Model is significantly higher compared with the growth rates mentioned for anaerobic bacteria in Chen and Hasminoto Model (Speece, 1996).

A good linear relationship was observed between methane gas productions obtained from the experiments carried out under six different HRTs and predicted methane gas productions in Modified Stover Kincannon Model ($y= 0.9568x+78.074$, $R^2 =0.97$) (See Figure 4.98). The linear relationship between observed and predicted effluent methane gas productions were found to be lower ($y=0.785x-602.45$, $R^2=0.83$) in Van der Meer and Heertjes Model compared to Modified Stover Kincannon Model (See Figure 4.99) Similarly, the linear relationship between predicted and observed effluent methane gas productions and predicted methane gas in Chen and Hasminoto Model was found to be lower ($y=3.5863x-4.236$, $R^2=0.52$) such as Van der Meer and Heertjes Model compared to Modified Stover Kincannon Model (See Figure 4.100). The regression coefficients between predicted and observed methane gas productions is higher in Stover Kincannon Kinetic ($R^2=0.97$). Therefore the observed and predicted methane gas productions in every HRTs were compared for three kinetic models (See Table 4.28). The HRT_S versus observed and predicted effluent methane gas productions showed that the predicted effluent methane gases are closer to the observed values when the calculated kinetic constants were placed into Stover-Kincannon Kinetic model**.**

Table 4.27 Comparison of kinetic constants for Modified Stover-Kincannon, Van der Meer and Heertjes and Chen and Hasminoto models for total and methane gas productions.

Model	**Kinetic constants**	**Values**	
		Methane gas production	**Total gas production**
Modified Stover-Kincannon Model	G_{max} (ml/l day)	-	1666.7
	G_B (mg/l day)	-	2.83
	M_{max} (ml/l day)	476.2	-
	M_B (mg/l day)	1.67	-
		$R^2=0.98$ $y= 0.0035x+0.0021$	$R^2=0.97$ $y=0.0017x+0.0006$
Chen and Hasminoto Model	B_{max} (l CH_4/kg COD removal)	0.283	-
	μ_{max} (day^{-1})	0.579	-
	k (dimensionless)	0.164	-
		$R^2=0.68$ $y=3.531x-5.0974$	-
Van der Meer and Heertjes Model	k_{sg} (ml CH_4/mg COD removed)	0.0947	-
		$R^2= 0.83$ $y=0.0947x+1069$	-

Table 4.28 Comparison of predicted and experimental results for total and methane gas productions in three different models.

HRT (day)	**Experimental**		**Predicted**			
			Modified Stover-Kincannon Model		**Van der Meer and Heertjes Model**	**Chen and Hasminoto Model**
	Total gas (ml/day)	**Methane gas (ml/day)**	**Total gas (ml/day)**	**Methane gas (ml/day)**	**Methane gas (ml/day)**	**Methane gas (ml/day)**
10.38	2160	980	2157	979	361	976
5.19	3420	1560	3936	1699	709	1805
3.4	5280	2370	5565	2299	1099	2541
2.4	8840	3360	7157	2826	1514	3017
1.5	9600	3552	9532	3558	2356	6864
1	12000	3800	11834	4197	3164	16080

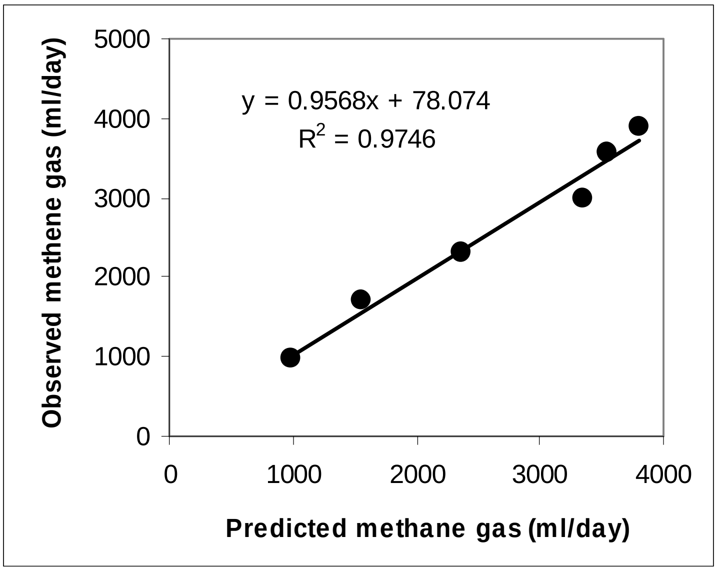


Figure 4.98 The observed and predicted daily methane gas productions for Modified Stover Kincannon Model.

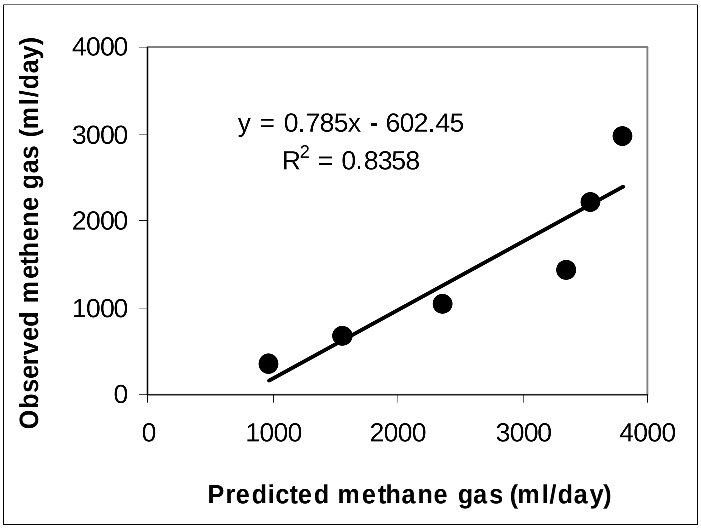


Figure 4.99 The observed and predicted daily methane gas productions for Van der Meer and Heertjes Model.

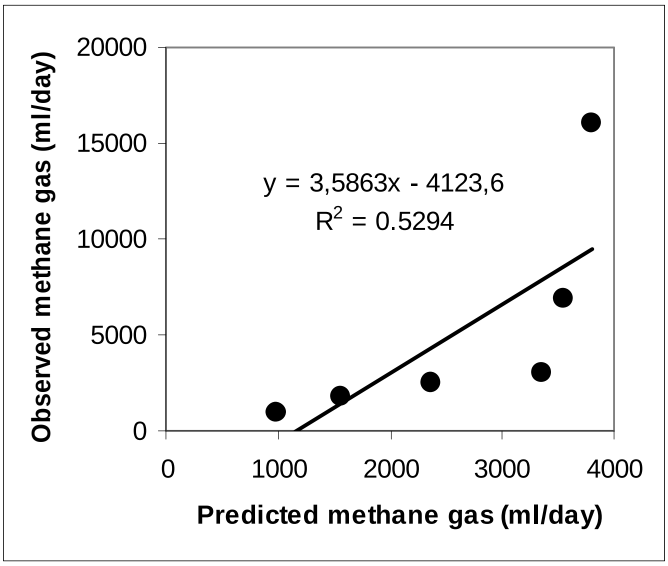


Figure 4.100 The observed and predicted daily methane gas productions for Chen and Hasminoto Model.

4.2.5.3 Kinetics of p-NP and COD Removal in ABR

In order to obtain the kinetic coefficient for different kinetic models the ABR reactor was operated with synthetic wastewater containing p-NP at six different HRTs through 185 days of the operation period**.** Monod**,** Grau first order, Grau second order, Modified Stover-Kincannon model, Zero order, First order, Second order kinetic models were applied to the experimental dates obtained from ABR reactor treating p-NP at six HRT. All kinetic coefficients calculated from the models are given in Table 4.29 with correlation coefficients. The kinetic data showed that Stover-Kincannon and Grau second order multicompenant substrate removal kinetics were more appropriate models than the other models for predicting the performance of the lab scale ABR reactor when the regression coefficients and kinetic coefficients were compared with each other. The effluent COD and p-NP concentrations were calculated using the Eq (4.19) and (4.20) for Modified Stover Kincannon kinetic. The effluent COD and p-NP concentrations were calculated using the Eq (4.21) and (4.22) in Grau second order kinetic model.

Modified Stover Kincannon kinetic

$$Se = Si - \frac{9.94 * Si}{9.7 + (Si / q_h)} \tag{4.19}$$

$$Ne = Ni - \frac{1.84 * Ni}{1.87 + (Ni / q_h)} \tag{4.20}$$

Grau second order kinetic

$$S_e = S_i (1 - \frac{1}{(0.97 + 0.32 / q_h)} \tag{4.21}$$

$$N_e = N_i (1 - \frac{1}{(1.02 + 0.042 / q_h)} \tag{4.22}$$

The yield coefficiency (Y) was higher compared to the death rate coefficient (k_d) in Monod kinetic model. Half saturation constant (K_S) was lower compared to initial COD concentration of 3000 mg/l. K_S values estimated from the Monod model is very lower (K_s=0.037 mg/l) than values reported by Pavlostathis & Giraldo-Gomez, (1991). Maximum substrate removal rate (R_{max}=33.11 gCOD/l day) in the Modified Stover-Kincannon model was higher than Grau first order (k_f=1.15 gCOD/l day) kinetic models. High COD utilization rate increase the reactor efficiency.

In order to test the validity of the Grau second order and Stover Kincannon kinetic models the results obtained from the experimental analysis (observed values) were compared with the values obtained from the models (predicted values). Table 4.29 gives the values obtained from the experimental analysis (observed values) and predicted values obtained from the Grau second order and Stover Kincannon kinetic. As can be seen from Table 4.29, the predicted values obtained from the Stover Kincannon model were very close to the experimental results than those obtained from Grau second order model (See Table 4.29).

A good linear relationship was observed and predicted effluent COD and p-NP concentrations obtained under six different HRTs in Modified Stover Kincannon Model ($y=1.045x-27.78$ and $R^2=0.97$, and $y=1.067x-0.069$ and $R^2=0.98$, respectively, for COD and p-NP) (See Figures 4.101 and 4.102). The linear relationship between observed and predicted effluent COD, p-NP concentrations in Grau second order kinetic ($y=1.098x-13.37$, $R^2=0.84$; $y=1.235x-0.624$ and, $R^2=0.84$ respectively for COD and p-NP**)** were found to be lower than that obtained from the Modified Stover- Kincannon model (See Figures 4.103 and 4.104). The regression coefficients between predicted and observed effluent COD and p-NP concentrations is higher in Stover Kincannon Kinetic ($R^2=0.97$).

In this study, the saturation constant (K_B) and maximum utilization rate (R_{max}) values were higher than those obtained by Işık & Sponza (2005) (R_{max}=7.5 g/l day, K_B=8.2 g/l day) in modified Stover-Kincannon model. High COD utilization rate (R_{max}) indicates high reactor efficiency in the ABR. But K_B and R_{max} values were found to lower in ABR reactor comparing to values obtained in AMBR

(R_{max}=33.1 g/l day, K_B=30.9 g/l day). The multicomponent substrate rate constant (k_S) value obtained in this study was very similar to those obtained in UASB reactor by Işık & Sponza, (2005) and Ubay, (1989) in Grau second order model (See Table 4.26).

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Table 4.28 Kinetic parameters of ABR reactor treating p-NP.

		COD removal		**p-NP removal**	
Kinetic models	**Kinetic parameters**	**Values**	**Regression coefficients (R^2)**	**Values**	**Regression coefficients (R^2)**
Monod	Y (gVSS/gCOD)	0.463	0.97	12.4	0.96
	k_d (day^{-1})	0.0041	0.97	0.003	0.96
	μ_{max} (day^{-1})	0.0013	0.68	0.07	0.79
	K_s (g/l)	0.037	0.68	0.24	0.79
Grau first order	p (l/day)	0.37	0.89	0.077	0.63
	k_f (g/l.day)	1.15	0.89	6.98	0.63
	k_f'(l/day)	0.027	0.89	0.164	0.63
Grau second order	k_s (l/day)	0.231	0.82	0.045	0.89
	a (day)	0.32	0.82	0.042	0.89
	b (dimensiones)	0.97	0.82	1.019	0.89
Modified Stover-Kincannon	K_B (g COD/l day)	9.7	0.99	1.87	1
	R_{max} (g COD/l day)	9.94	0.99	1.84	1
Zero order	k_0 (mg /l day)	81.05	0.61	0.0003	0.29
First order	k_1 (day^{-1})	0.37	0.89	0.0019	0.97
Second order	k_2 (l /mg day)	6.4	0.87	0.019	0.97

Table 4.29 Comparison of predicted and experimental results for Modified Stover Kincannon and Grau second order kinetic model.

Effluent COD and p-NP concentration (mg/l)						
HRT (day)	**Experimental (Observed)**		**Stover-Kincannon (Predicted)**		**Grau-second order (Predicted)**	
	COD (mg/l)	**p-NP (mg/l)**	**COD (mg/l)**	**p-NP (mg/l)**	**COD (mg/l)**	**p-NP (mg/l)**
10.38	25	1.9	20	1.6	3	1.8
5.19	92	2.2	127	2.0	100	2.1
3.4	122	2.2	210	2.3	189	2.4
2.4	382	2.5	327	2.7	298	2.9
1.5	510	3.5	542	3.5	560	3.5
1	810	4.9	791	4.5	690	4.5

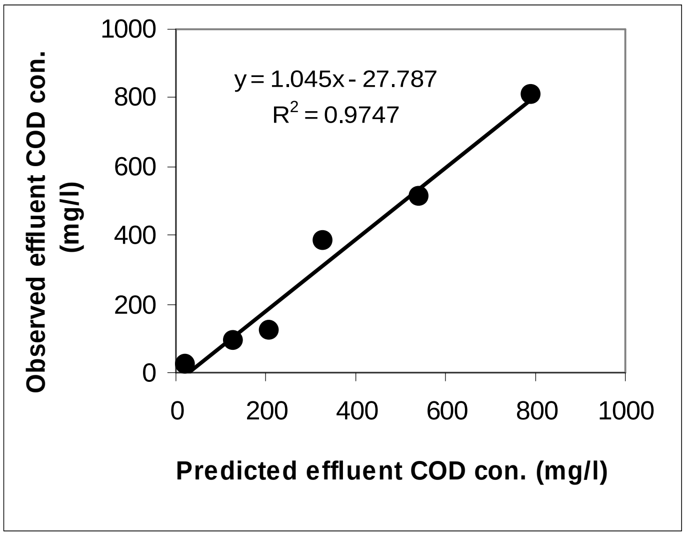

Figure 4.101 The observed effluent COD and predicted effluent COD concentrations in Stover-Kincannon Kinetic model.

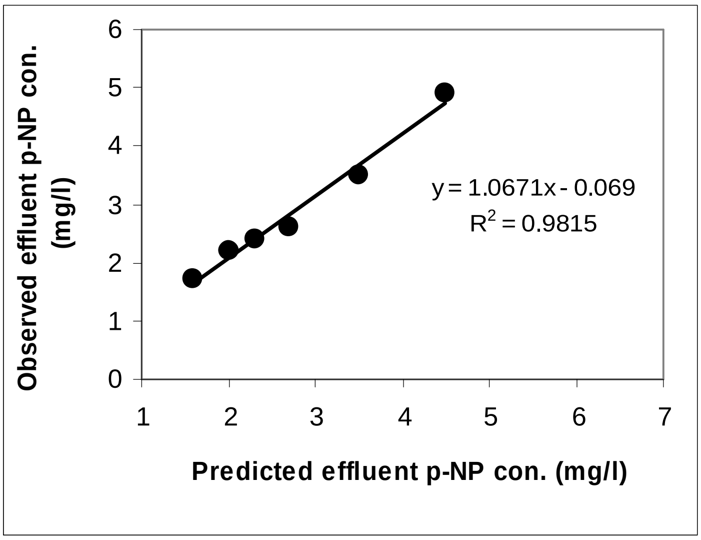

Figure 4.102 The observed effluent p-NP and predicted effluent p-NP concentrations in Stover-Kincannon Kinetic model.

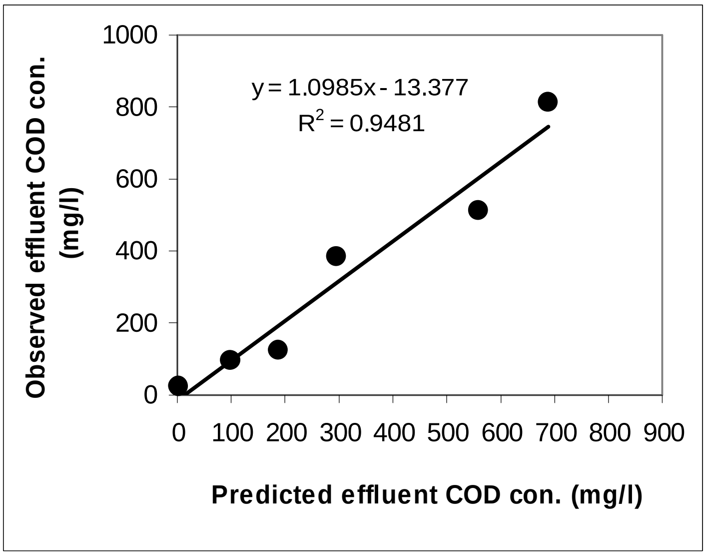

Figure 4.103 The observed effluent COD and predicted effluent COD concentrations in Grau second order kinetic model.

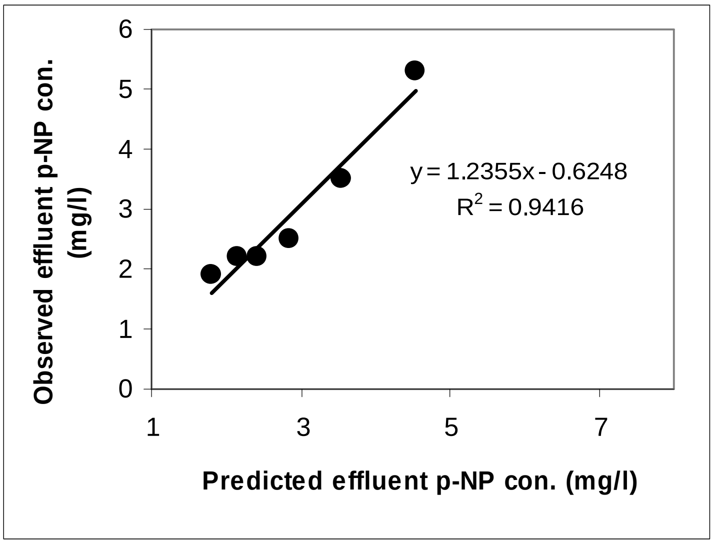

Figure 4.104 The observed effluent p-NP and predicted effluent p-NP concentrations in Grau second order kinetic model.

4.2.5.4 Biogas Production Kinetics

4.2.5.4.1 Modified Stover-Kincannon Model. In order to determine the kinetic coefficients (G_{max} and M_{max}) Eqs. (3.50) and (3.51) were used to calculate the total and methane gas productions in ABR. The reciprocal of specific gas production (1/G) versus reciprocal of applied substrate loading rates (1/OLR) were plotted in order to determine the kinetic constants relevant to total and methane gas productions, respectively. The intercept and slope of the line result in $1/G_{max}$ and K_B/G_{max}, respectively. The maximum specific total gas production rate, G_{max}, and proportionality constant, G_B, were found to be 14285 ml/l.day and 27.12 (dimensionless), respectively (R^2=0.98, y=0.0019x+0.00005). The maximum methane gas production rate, M_{max}, and proportionality constant, M_B, were found to be 400 ml/l.day and 3.4 (dimensionless), respectively **(**R^2=0.98, y= 0.0085x+ 0.0028). The specific total gas and methane gas production rates were predicted using Eq. 3.50 and 3.51 as follows:

$$\frac{1}{G}=\frac{27.14}{14285}\times\frac{1}{OLR}+\frac{1}{14285} \tag{4.19}$$

$$\frac{1}{M}=\frac{3.4}{400}\times\frac{1}{OLR}+\frac{1}{400} \tag{4.20}$$

G is specific total gas production rate (ml/day), while M is specific methane gas production rate (ml/day). OLR is organic loading rate (g/l.day).

4.2.5.4.2 Van der Meer and Heertjes Model. To describe the kinetic for gas production, equation (17) was used. Kinetic constant, k_{sg}, was determined empirically from the slope of the line plotted between $Q(S_i\text{-}Se)$ and CH_4 (R^2=0.54, y= 0.0452x+1.164). The kinetic constant of gas production (k_{sg}) was found as 0.045 ml CH_4/mg COD removed.

The methane gas production in ABR can be predicted as follow:

$$V=0.045\ Q\ (S_i-S_e) \tag{4.21}$$

The methane gas productions were found between 393 and 2925 ml/day at HRTs varied from 10.38 to 1 day according to Eq (4.16).

4.2.5.4.3 *Chen and Hasminoto Model.* The ultimate methane yield (B_{max}) was calculated as 0.115 l CH_4/g COD removed from the intercept plotted between methane yield and 1/HRT using Eq. (21) ($R^2=0.69$, $y= 0.0573x+0.1159$). B_{max} value obtained from the study performed by Setiadi, Husaini & Djajadiningrat, (1996) was 0.33 l CH_4/g COD removed in an ABR treating palm oil mill effluent. This result is significantly higher than the Y_{max} value obtained from the our study. This showed that the partial inhibion of methanogenic microorganism by p-NP.

The maximum specific growth rate (μ_{max}) and k was determined graphically by plotting the term $B_{max}/(B_{max}-B)$ versus HRT. The slope of the straight line is equal to μ_{max}/k and the intercept is equal to $(1-1/k)$. The maximum specific growth rate (μ_{max}) was found as 0.317 day^{-1} and Chen and Hasminoto kinetic constant (k) was found as 0.0647 (dimensionless) ($R^2=0.23$, $y= 0.204x+3.15$). In this model, methane yield and maximum specific growth rate of methanogens affected by the hydraulic retention time and Chen and Hasminoto kinetic constant.The methane gas productions can be predicted as follows with re-aranged Eq 3.56.

$$B = B_{\max} - \frac{B_{\max}.k}{q_h * m_{\max} + k - 1} \tag{4.22}$$

$$B = 0.115 - \frac{0.115 \times 0.0647}{q_h * 317 + 0.0647 - 1} \tag{4.23}$$

4.2.5.4.4 Evaluation of the Biogas Kinetic Models

The kinetic constants calculated from the biogas kinetic models are summarized in Table 4.30 for ABR reactor. The results obtained for observed and predicted total and methane gas productions are given in Table 4.31. The total and methane gas productions predicted from the Modified Stover-Kincannon model was very close to the experimental data compared to the other two models at six different HRTs. The linear relationships showed that the regression coefficient is higher in Modified Stover-Kincannon model (R^2=0.98) compared to the other two models (See Table 4.30).

A good linear relationship was observed between methane gas productions obtained from the experiments carried out under six different HRTs and predicted methane gas productions in Modified Stover Kincannon Model (y= 1.114x-206.5, R^2 =0.95) (See Fig. 4.105). The linear relationship between observed and predicted effluent methane gas productions were found to be lower (y=0.625x-353.9, R^2=0.83) (See Figure 4.106) in Van der Meer and Heertjes Model compared to Modified Stover Kincannon Model. Similarly, the linear relationship between predicted and observed effluent methane gas productions and predicted methane gas calculated in Chen and Hasminoto Model was found to be lower (y=0.052x+34.16, R^2=0.37) than those obtained from Modified Stover Kincannon Model (See Figure 4.107). As a result it was showed that the regression coefficients between predicted and observed methane gas productions is higher in Stover Kincannon Kinetic (R^2=0.97). Therefore the observed and predicted methane gas productions in every HRTs were compared for three kinetic models (See Table 3.31). The observed and predicted methane gas productions versus HRTs showed that the predicted methane gases are closer to the observed value in Stover-Kincannon Kinetic model (See Figure 4.108).

Table 4.30 Comparison of kinetic constants for Modified Stover-Kincannon, Van der Meer and Heertjes and Chen and Hasminoto models for total and methane gas productions.

Model	**Kinetic constants**	**Values**	
		Methane gas production	**Total gas production**
Modified Stover-Kincannon Model	G_{max} (ml/l.day)	-	14285
	G_B (dimensionless)	-	27.14
	M_{max} (ml/l.day)	400	-
	M_B (dimensionless)	3.4	-
		R^2=0.98 y= 0.0085x+0.0028	R^2=0.98 y=0.0019x+0.00007
Chen and Hasminoto Model	B_{max} (l CH_4/kg COD removed)	0.115 (or 0.009 l CH_4/g VSS added)	-
	μ_{max} (day $^{-1}$)	0.317	-
	k (dimensionless)	0.0647)	-
		R^2=0.23; y=0.204x+3.15	-
Van der Meer and Heertjes Model	k_{sg} (ml CH_4/mg COD removed)	0.095	-
		R^2= 0.83 y=0.0956x+0.199	-

Table 4.31 Comparison of predicted and experimental results for total and methane gas productions in three different models.

HRT (day)	**Experimental total and methane gas (ml/day)**		**Predicted total and methane gas (ml/day)**			
			Modified Stover-Kincannon Model		**Van der Meer and Heertjes Model**	**Chen and Hasminoto Model**
	Total	**Methane**	**Total gas**	**Methane**	**Methane**	**Methane**
10.38	2160	944	2110	938	393	135
5.19	3600	1510.4	4174	1731	753	115
3.4	7200	2712	6394	2438	980	138
2.4	9600	3293.92	8951	3212	1450	141
1.5	15360	4403.2	13737	4394	2000	149
1	17280	4632	20011	5547	3200	450

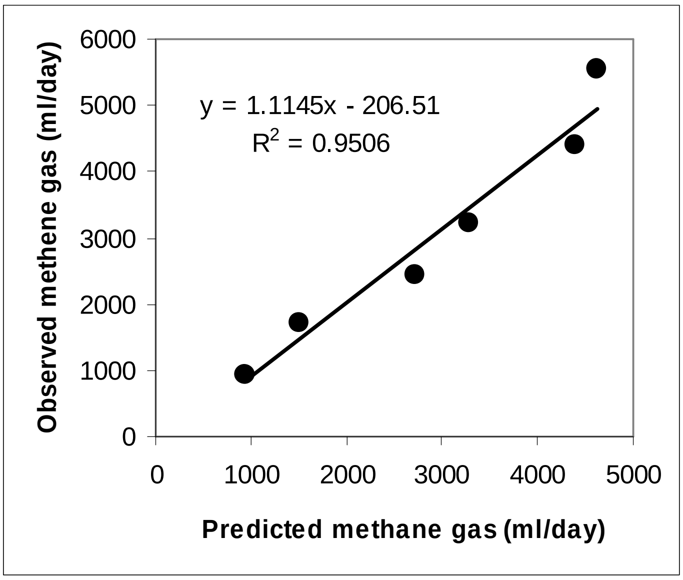

Figure 4.105 The observed and predicted daily methane gas productions for Modified Stover Kincannon Model.

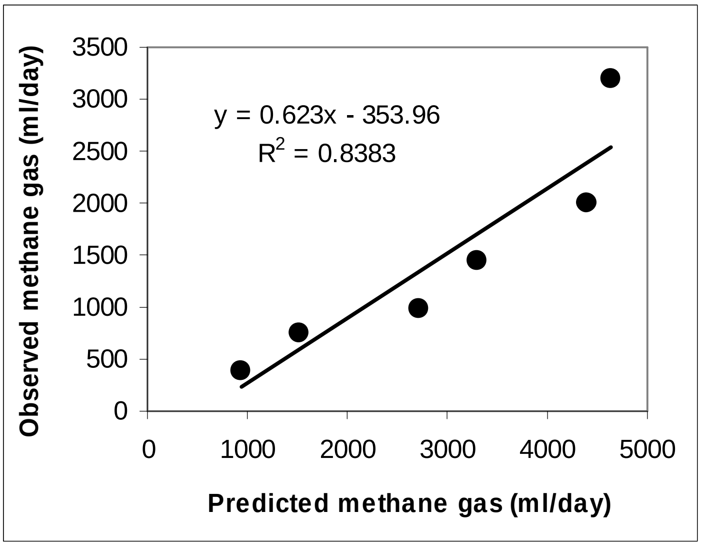

Figure 4.106 The observed and predicted daily methane gas productions for Van der Meer and Heertjes Model.

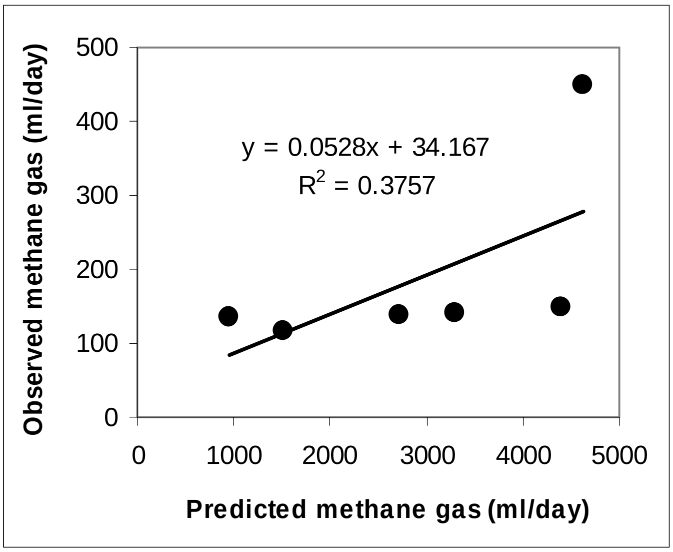

Figure 4.107 The observed and predicted daily methane gas productions for Chen and Hasminoto Model.

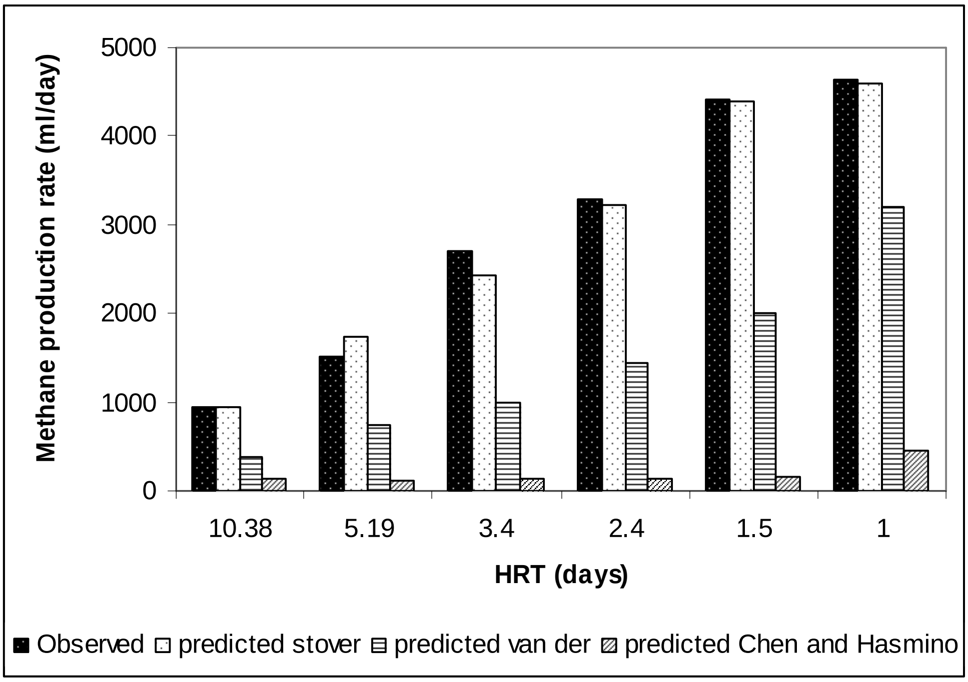

Figure 4.108 Comparison of the observed and predicted methane gas productions for Modified Stover Kincannon Model, Van der Meer and Heertjes Model, Chen and Hasminoto Models.

4.2.6 Process Economy

4.2.6.1 Process Economy of AMBR treating p-NP

The CH_4 produced from the AMBR treating p-NP of 40 mg/l at HRTs of 1 day is equal to 3.78 l/day (0.00378 m^3/day). The electric energy produce from 1 m^3 CH_4 is equel to 11.04 kWh. The energy yield is equal to 0.042 kW/day (42 W per day).

The engine electricity concumed from the apparatus are as follows:

1) The mecanic mixer consumes 20 Wh of electric energy per hours. Since the mixer was operated 15 min. in a hour the total electric energy used in this apparatus in aproximately 5 Wh.

2) The peristaltic pump consumes 20 Wh of electric energy per hours. Since the peristaltic pump was operated 15 min. in a hour the total electric energy used in this apparatus in aproximately 5 Wh.

3) The air pump in the aeration tank of the CSTR reactor consume 10 Wh of electric energy per day.

4) The heater in the AMBR reactor consumes 10 Wh of electric energy per day.

The total consumed energy is equal to 720 Wh per day.

If the enegy obtained from the AMBR reactor is compared with the concumed energy the electricity obtained from the methane is lower (42 W per day) than that consumed energy (720 W per day). However, it is important to note that the COD concentration used in the AMBR reactor is low (COD=3000 mg/l). If high COD concentration will used in this reactor (10.000-30.000 mg/l) the AMBR reactor will produce 140 W or 420W per day of electric energy. This energy will be used to oppose the total energy expenses originated from the apparatus used in this study.

4.2.6.2 Process Economy of ABR treating p-NP

The CH_4 produced from the ABR treating p-NP of 85 mg/l at HRTs of 1.5 day is equal to 5.12 l/day (0.00512 m^3/day). The electric energy produce from 1 m^3 CH_4 is equel to 11.04 kWh. The energy yield is equal to 0.056 kW/day (56W per day).

The engine electricity concumed from the apparatus are as follows:

1) The peristaltic pump consumes 20 Wh of electric energy per hours. Since the peristaltic pump was operated 15 min. in a hour the total electric energy used in this apparatus in aproximately 5 Wh.

2) The air pump in the aeration tank of the CSTR reactor consumes 10 Wh of electric energy per day.

3) The heater in the ABR reactor consumes 10 Wh of electric energy per day.

The total consumed energy is equal to 600 Wh per day.

If the enegy obtained from the ABR reactor is compared with the concumed energy the electricity obtained from the methane is lower (56 W per day) than that consumed energy (600 W per day). However, it is important to note that the COD concentration used in the ABR reactor is low (COD=3000 mg/l). If high COD concentration will used in this reactor (10.000-30.000 mg/l) the ABR reactor will produce 187 W or 560W per day of electric energy. This energy will be used to oppose the total energy expenses originated from the apparatus used in this study.

CHAPTER FIVE
CONCLUSIONS

5.1 Conclusions

The p-NP and NB concentrations caused 50% decreases in the methanogenic activity (decrease of methane gas production) (IC_{50}) were calculated as 27.7 mg/l and 109 mg/l, respectively.

Removal of p-NP with abiotic and volatilization ways and accumulation of p-NP into sludge test results showed that 1.9 % of p-NP was adsorbed by anaerobic biomass; 1.5 % of p-NP was removed by volatilization and 0.06 % of p-NP was accumulated into granular sludge. Therefore these mechanisms are not significant in biological removal of p-NP.

Abiotic and volatilization and accumulation test results for NB showed that 1.2 % of NB was adsorbed by anaerobic biomass; 1.3 % of NB was removed by volatilization; 0.04 % of NB was accumulated into granular sludge, which these mechanisms are not significant in biological removal of NB.

AMBR reactor reached steady-state conditions after an operation period of 30 days. After this operation time, COD removal efficiency and methane gas percentage remained constant approximately 93% and 52%, respectively, through continuous operation.

ABR reactor reached steady-state conditions after an operation period of 45 days. The COD removal efficiency was found as 94% after 45 days of the start-up period. The daily methane gas production and methane percentage remained stable at 1500 ml/day and 60%, respectively.

Toxic p-NP and NB transformed less toxic intermediate products after anaerobic treatment.

5.1.1 The Removal of p-NP in AMBR and AMBR/CSTR Reactor System

1. The maximum p-NP loading rate and p-NP concentration introduced in AMBR were found as 3.85 g/m^{3}day and 40 mg/l, respectively. COD and p-NP removal efficiencies were 92 % and 93% at this loding rate and p-NP concenteration, respectively. After this p-NP loading rate and p-NP concentration, the COD and p-NP removal efficiency decreased. The maximum COD and p-NP (E = 90–92% and E=92–95%) removal efficiencies were observed at HRTs varying between 3.4 and 10.38 days. The COD and p-NP removal efficiencies were 91% and 92% at a HRT of 3.4 days, respectively. It is important to note that. Therefore the AMBR reactor can be operated at a HRT of 3.4 days since the removal efficiencies exhibits similaritias with a HRT of 10.38 days. This result reduces the reactor volume resulting in the process volume economy.

2. p-NP was transformed to p-AP with efficiency near to 100% in anaerobic phase. Furthermore, phenol was observed in the compartments and in the effluent of anaerobic reactors as intermediate metabolite. The p-AP was partially mineralized to phenol. The 2-6-bis(1,1-dimethylethy)-4-methyl phenol was not detected in the samples taken from the compartments and from the effluent of AMBR. The generation of ammonia (NH_4- N) was observed during the anaerobic degradation of p-AP in AMBR. The p-AP, phenol and NH_4-N produced in the anaerobic phase converted to nitrite (NO_2), nitrate (NO_3) and CO_2 as end products under aerobic conditions.

3. The maximum total, methane gas productions and methane percentage were found about 2300 ml/day, 1300 ml/day and 56%, respectively at a p-NP loading rate of 4.81 g/m^{3}day. The maximum total and methane gas productions were 12.25 l/day and 3.8 l/day, respectively, at a HRT of 1 day. The optimum HRTs for maximum methane gas productions (43–47%) varied between 10.38 and 3.4 days.

4. The COD and p-NP removal efficiencies were higher in the first compartment than the other two compartments. The COD and p-NP removal efficiencies

were approximately 80% and 90%, respectively in the initial compartment. Small COD removals (E=between 13 % and 46%) occurred in the compartment 2 and the remaining fraction of the influent COD was removed with removal efficiencies varying between 9 % and 22% in compartment 3.

5. TVFA concentrations in the effluent increased with increasing p-NP loading rates and decreasing HRTs. The TVFA concentration in the effluent increased from 23 mg/l to 177 mg/l as HRT decreased from 10.38 to 1 day. TVFA concentrations were high in the first compartment compared to the other compartments. However, TVFA concentration decreased throughout compartments. TVFA concentration in the effluent were below 480 mg/l even was studied at p-NP loading rate as high as 9.13 g/m^{3}day. TVFA/Bic.Alk. ratios in the effluent and in the compartments were lower than 0.4 in increasing p-NP loading rates (from 0.96 to 9.63) and decreasing HRTs (from 10.38 days to 1 day). This indicates the stability of AMBR reactor**.**

6. The COD removal efficiencies were up to 90% until a p-NP loading rate of 8.18 g/m^3 day. The p-NP removal efficiencies were found between 97% and 99% at all p-NP loading rates in sequential AMBR/CSTR reactor system. Overall total COD and p-NP removal efficiencies decreased from 99% to 94% and from 96% and 92%, respectively, as the HRT decreased from 17.3 days to 1.7 days in sequential anaerobic AMBR/aerobic CSTR reactor system. The maximum COD and p-NP removal efficiencies were found as 98% and 97%, respectively at a HRT of 17.3 days in sequential anaerobic AMBR/aerobic CSTR reactor system.

7. In Lumistox toxicity study, the wastewater containing 40 mg/l of p-NP was found to be toxic (% inhibition (H) =98%). After anaerobic treatment, % inhibition (H) value decreased to 58.1% in the effluent of AMBR reactor. After aerobic treatment, toxicity decreased from 58% to 19 % in aerobic effluent. The aerobic reactor effluent was moderate toxic exhibiting the possible toxicity. In *Daphnids* toxicity test, After 24 h of incubation time, IC_{50} value of the feed wastewater containing p-NP of 40 mg/l (the concentration inhibited 50% of *Daphnia magna*) was found as 4 mg/l in feed

wastewater diluted 10 times. This showed that the feed wastewater was toxic for *Daphnids*. IC_{50} value of aerobic effluent (50% inhibition of *Daphnids)* was found as 21.6 mg/l in diluted wasrwater at a ratio of 4/5. This showed that anaerobic reactor decreased the toxicity of the influent and produced less toxic intermediates than the influent.

5.1.2 The Removal of p-NP in ABR and ABR/CSTR Reactor System

1. The maximum p-NP loading rate and p-NP concentration introduced in ABR were found as 8.32 g/m^{3}day and 80 mg/l, respectively. COD and p-NP removal efficiencies were 92 % and 93% at this loding rate and p-NP concenteration, respectively. After this p-NP loading rate and p-NP concentration, the COD and p-NP removal efficiency decreased. The COD (E=95-99%) and p-NP (E=97-98%) removal efficiencies exhibited a good performance until HRT of 3.4 days. If the ABR reactor operates at a HRT of 3.4 days, the reactor volume will reduce, resulting in the process volume economy.

2. The optimum p-NP loading rate and p-NP concentration were found as 8.32 g/m^{3}day and 85 mg/l, respectively, for maximum COD (E=92%) and p-NP (E=99%) removal efficiencies. Maximum COD (E >95%) and p-NP (E=97%) removal efficiencies were found at a HRT of 3.4 days in ABR.

3. Optimum total, methane gas and methane percentage were found as 2300 ml/day, 1300 ml/day and 50%, respectively, as the p-NP loading rates varied between 0.98 g/m^{3}day and 8.32 g/m^{3}day. The total gas and methane gas production rates increased from 2.2 to 17.7 l/day and from 0.9 to 4.3 l/day, respectively as the HRT decreased from 10.38 days to 1 day. The methane content of biogas decreased from 39% to 16% as the HRT decreased from 10.38 days to 1 day. Maximum methane percentages (39%) were found at HRTs varied between 10.38 days and 3.4 days.

4. pH values in compartments were between optimum values (pH 6.5 and 8.3) for anaerobic degradation at increasing p-NP loading rates and decreasing

HRTs. TVFA concentrations in the effluent were 0 mg/l at all p-NP loading rates and until a HRTs of 3.4 days. TVFA/Bic.Alk. ratios in the effluent and in the compartments of ABR were lower than 0.4. These results indicated the stability of ABR reactor at increasing p-NP loading rate and decreasing HRTs.

5. The overall COD removal efficiencies were up to 90% until a p-NP loading rate of 48.5 g/m^3.day in sequential ABR/CSTR reactor system. The p-NP removal efficiencies increased from 97 % to 99% as the p-NP loading rates increased from 0.97 to 8.32 g/m^{3}day in sequential ABR/CSTR reactor system. The overall COD and p-NP removal efficiencies decreased from 99% to 86% and from 99% to 93%, respectively, when the HRT were decreased from 13.6 to 1.3 day in sequential ABR/CSTR reactor system.
6. In Lumistox toxicity test in ABR/CSTR reactor system, the wastewater containing 85 mg/l of p-NP was found to be toxic (% inhibition (H)= 99%). After anaerobic treatment, the effluent toxicity decreased to 62 %. After aerobic treatment, toxicity decreased from 62% to 28 % in aerobic effluent. The aerobic reactor effluent was moderate toxic exhibiting possible toxicity.

5.1.3 The Removal of NB in AMBR and AMBR/CSTR Reactor System

1. The maximum NB loading rate and NB concentration introduced in AMBR were found as 3.85 g/m^{3}day and 60 mg/l, respectively. COD and p-NP removal efficiencies were 94 % and 100% at this loding rate and p-NP concenteration, respectively. After this NB loading rate and NB concentration, the COD removal efficiency decreased. But NB removal efficiency not changed (E=100%). The COD removal efficiency exhibited a good performance until HRT of 5.19 days. COD removal efficiency was 92% at HRT of 5.19 days. NB removal efficiencies were 100% at all HRTs.
2. The maximum total gas, methane gas productions and methane percentage were 2.8 l/day, 1.3 ml/day and 45 %, respectively at a NB loading rate of 5.78 g/m^{3}day. Maximum total and methane gas production and methane

percentage were observed as 5.6 l/day and 2.6 l/day and 46% at a HRT of 3.5 days, respectively.

3. NB was reduced to aniline, in the first step, under anaerobic conditions. In the second step, aniline was mineralized to catechol, then 2-Hydroxymuconic semialdehyde via catechol-2,3-dioxygenase and the end is joined to TCA (tricarboxylic acid) cycle under aerobic conditions.

4. The pH values in the effluent and in the compartments of AMBR were between the optimum pH values for anaerobic treatment at the increasing NB loading rate and decreasing HRTs. TVFA concentrations in the effluent of AMBR was zero except for NB loading rates of 28.9 and 38.54 g/m^{3}day. The TVFA concentrations in the effluent were 11 mg/l and 17 mg/l at aforementioned loading rates, respectively. TVFA concentrations in effluent of AMBR measured as zero until a HRT of 3.5 days. The TVFA concentrations in effluent of AMBR increased from 150 mg/l to 350 mg/l, when the HRTs were decreased from 2 days to 1 day. TVFA/Bic.Alk. ratios were found below 0.4 in all compartments and in the effluent of AMBR, which indicates the stability of AMBR.

5. Overall COD removal efficiencies varied between 93% and 97 % at NB loading rates varying between 1.9 and 38.5 g/m^{3}day in sequential anaerobic AMBR/aerobic CSTR reactor system. COD removal efficiencies in the total system varied between 93% and 98% at all HRTs, respectively. The removal of NB was 100 % in ABR in sequential ABR/CSTR reactor system.

5.1.4 The Removal of NB in ABR and ABR/CSTR Reactor System

1. The maximum NB loading rate and NB concentration introduced in ABR were found as 12.5 g/m^{3}day and 130 mg/l, respectively. COD and p-NP removal efficiencies were 92 % and 100% at this loding rate and NB concenteration, respectively. In HRT study, the maximum COD removal efficiencies were found as approximately 94 % at HRTs varied between 2.5 days to 10.38 days. Therefore the ABR reactor can be operated at a HRT of

2.5 days. This result reduces the reactor volume resulting in the process volume economy.

2. The maximum total, methane gas productions and % methane production were about 1300 ml/day and 3300 ml/day and 50 %, respectively, until a NB loading rate of 20.23 g/m^{3}day. The maximum total gas, methane gas productions were found at a HRT of 1 day. Methane gas percentages decreased from 43% to 29% as the HRT decreased from 10.38 days to 1 day, respectively.

3. The effluent pH values were between 7.2 and 7.9 at all NB loading rates. The effluent pH values remained stable between 7.2 and 7.4 in ABR at all HRT. TVFA concentration was zero in the effluent with the exception of a NB loading rate of 67.43 g/m^{3}day. The TVFA concentration was measured as 32 mg/l in the effluent samples of ABR reactor for a NB loading rate of 67.43 g/m^{3}day**.** TVFA concentration in the effluent of ABR increased from 0 mg/l to 480 mg/l as HRT decreased from 10.38 days to 1 day. However TVFA/Bic.Alk. ratios were below 0.3 at all HRT. This shows the stability of ABR reactor.

4. Overall COD removal efficiencies varied between 95% and 99% in sequential ABR/CSTR reactor system at NB loading rates varying between 1.9 and 67.4 g/m^{3}day. 96-98% COD and 100% NB removal efficiencies were measure at HRTs varying between 1.67 and 17.3 days in sequential anaerobic/aerobic system.

5. In Lumistox toxicity test of ABR/CSTR reactor system treating NB of 130 mg/l at HRT of 3.5 days, the feed wastewater containing a NB concentration of 130 mg/l was found to be toxic (% inhibition (H)= 87%). After anaerobic treatment, the effluent toxicity value decreased to 48 % in ABR reactor. After aerobic treatment, toxicity decreased from 48 % to 18 % in aerobic reactor. The aerobic reactor effluent was moderately toxic exhibiting the possible toxicity.

5.1.5 Determine of Kinetic Constant for AMBR and ABR Reactors Treating p-NP

1. Modified Stover-Kincannon substrate removal kinetic was found to be the appropriate kinetic model for predicting the performance of the lab scale AMBR reactor. K_B and R_{max} was 29.498 gCOD /l.day and 33.55 gCOD /l.day, respectively with high regression coefficient (R^2=1) for COD. Similarly, the R_{max} and K_B values for p-NP were obtained as 0.407 g p-NP/lday and 0.428 g p-NP/lday, respectively, with high regression coefficient (R^2=1).

2. Appropriate biogas kinetic model for AMBR was found to be Modified Stover-Kincannon model. The maximum specific total gas production rate, G_{max}, and proportionality constant, G_B, were obtained found to be 1666.7 ml/l.day and 2.83 (mg/lday), respectively (R^2 = 0.97). The maximum methane gas production rate, M_{max}, and proportionality constant, M_B, were 476.2 ml/l day and 1.67 mg/l day, respectively (R^2=0.98).

3. Appropriate substrate kinetic model for ABR was found to be Modified Stover-Kincannon kinetic model. K_B and R_{max} was 9.7 gCOD /l day and 9.94 gCOD /l day (R^2=1), respectively. Similarly, the R_{max} and K_B values for p-NP were obtained as 1.84 g p-NP/l day and 1.87 g p-NP/l day, respectively, with high regression coefficient (R^2=1).

4. Appropriate biogas kinetic model for ABR was found to be Modified Stover-Kincannon model. The maximum specific total gas production rate, G_{max}, and proportionality constant, G_B, were found to be 14285 ml/l.day and 27.12 (dimensionless), respectively (R^2=0.98). The maximum methane gas production rate, M_{max}, and proportionality constant, M_B, were 400 ml/l.day and 3.4 (dimensionless), respectively (R^2=0.98).

5.2 Recommendation

AMBR and ABR reactors are high rates compartmentalised reactors, containing high concentration of biomass. The results of this study showed that these reactors can be used effectively to treat the toxic wastewaters containing p-NP and NB. Since no more studies were performed with these reactors it can be recommended to utilization in a textile, dyes, chemical industrial containing toxic substances since in these reactors high COD and toxicant removal efficiencies was obtained. Furthermore, the utilization of these reactors can be recommended in the treatment of olive oil wastewaters as pretreatment plant or following the chemical treatment. On the other hand it can be used in the treatment of wastewater containing high COD concentrations and organic loading. This could be increase the methane production and methane percentage of the reactors. Increases in methane gas productions will be improved the electrical energy obtained from the CH_4 gas. This energy could be used in the expenses of the reactors such as mixer and pumps.

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APPENDICES

Nomenclature

a	Si / (ks *X) (day)
b	constant (dimensionless)
B	actual methane yield (l CH_4/ g COD)
B_{max}	ultimate methane yield (l CH_4/g COD)
G	specific gas production rate (ml /l day)
G_B	proportionality constant (mg/l day)
G_{max}	maximum specific gas production rate (ml/l day)
k	Chen and Hasminoto kinetic constant (dimensionless)
k_0	zero order kinetic constant (mg/l day)
k_1	first order kinetic constant (day^{-1})
k_2	second order kinetic constant (l/mg day)
K_B	saturation value constant (g COD/l day)
k_d	endogenous decay coefficient (day^{-1})
k_f	kf’ *X (mg/l.day)
$k_{f'}$	first order substrate removal kinetic constant (day^{-1})
k_s	Grau second-order substrate removal rate constant (day^{-1})
K_s	half saturation concentration (mg/ l)
k_{sg}	Van der Meer and Heertjes kinetic constant (ml CH_4/mg COD),
M	specific methane production rate (ml/l day)

M_B	proportionality constant (mg/l day)
M_{max}	maximum specific methane gas production rate (ml/l day)
N_e	effluent p-NP concentration (mg/l)
N_i	influent p-NP concentration (mg/l)
p	k_f/S_0 (day^{-1})
Q	influent flow rate (l/ day)
Q_e	flow rate of the effluent (l/day)
Q_W	flow rate of waste sludge (l/day)
R_{max}	maximum utilization rate (g COD/l day)
S_e	effluent substrate concentration (mg/l)
S_i	influent substrate concentration (mg/l)
V	reactor volume (l)
X_e	concentration of biomass in the effluent (g/l)
X_i	concentration of biomass in the influent (g/l)
X_r	concentration of biomass in the reactor (g/l)
X_W	microorganism concentration in waste sludge (g/l)
Y	growth yield coefficient (g VSS / g COD)
β	contais kinetic parameter (g COD/g biomass).
θc=SRT	sludge retention time (day)
θh =HRT	hydraulic retention time (day)
μ, μ_{max}	specific growth rate,maximum specific growth rate (day^{-1})

Abbreviations

ABR	Anaerobic Baffled Reactor
AMBR	Anaerobic Migrating Blanked Reactor
CSTR	Continuously Stirred Tank Reactor
HRT	Hydraulic Retention Time
SRT	Solid Retention Time
OLR	Organic Loading Rate
COD	Chemical Oxygen Demand
TSS	Total Suspended Solids
VSS	Volatile Suspended Solids
TVFA	Total Volatile Fatty Acid
Bic.Alk.	Bicarbonate Alkalinity
SMA	Specific Methanogenic Activity
HPLC	High Performance Liquid Chromatograpy
GC	Gas Chromotography
UV-VIS	Ultraviolet-Visible
mUA	Mili Amper Unit
SRB	Sulphate Reducing Bacteria