

**COMPARATIVE STUDY OF SEQUENCING BATCH
BIOFILM REACTOR AND SEQUENCING BATCH
REACTOR IN *p*-NITROPHENOL REMOVAL**

GOH CHIN PING

UNIVERSITI SAINS MALAYSIA

MAY 2008

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REACTOR IN *p*-NITROPHENOL REMOVAL**

by

GOH CHIN PING

**Thesis submitted in fulfillment of the
requirements for the degree of
Master of Science**

MAY 2008

ACKNOWLEDGEMENTS

I would particularly like to express my thanks to my main supervisor, Professor Lim Poh Eng, my co-supervisors Associate Professor Dr. Seng Chye Eng and Dr. Ahmad Ngilmi Ahmad Sujari for their invaluable guidance and advice. I wish to express my sincere thanks and gratitude to my parents for their support. I also owe a special thanks to Mr. Yee, Mr. Ong and Mr. Aw Yeong for their help during the research. Lastly, but not least, I wish to extend a special thank to my wife Loh Wai Hwan for her encouragement.

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ABBREVIATIONS

AN	Ammoniacal Nitrogen
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
GC-MS	Gas Chromatography-Mass Spectroscopy
HRT	Hydraulic Retention Time
HPLC	High Pressure Liquid Chromatography
MLSS	Mixed Liquor Suspended Solid
MLVSS	Mixed Liquor Volatile Suspended Solid
NO_2^- - N	Nitrite nitrogen
NO_3^- - N	Nitrate nitrogen
NO_x^- - N	Oxidized nitrogen
PNP	<i>p</i> -Nitrophenol
PTFE	Polytetrafluoroethylene
SBR	Sequencing Batch Reactor
SBBR	Sequencing Batch Biofilm Reactor
SEM	Scanning Electron Microscope
SND	Simultaneous Nitrification and Denitrification
SOUR	Specific Oxygen Utilization Rate
SRT	Sludge Retention Time
SS	Suspended Solid
SV_{30}	Settled Sludge Volume
SVI	Sludge Volume Index
UV-VIS	UV-Visible

**KAJIAN PERBANDINGAN REAKTOR BIOFILEM KELOMPOK
BERTURUTAN DAN REAKTOR KELOMPOK BERTURUTAN DALAM
PENYINGKIRAN *p*-NITROFENOL.**

ABSTRAK

Objektif kajian ini adalah untuk: (1) membandingkan biopenguraian *p*-nitrofenol (PNP) di bawah operasi reaktor biofilem kelompok berturutan (SBBR) dan reaktor kelompok berturutan (SBR) dan (2) mengenalpasti laluan biopenguraian PNP dalam sistem enapan aktif kultur campuran. Tiga buah reaktor 'plexiglass' yang serbasama dioperasikan dengan tempoh PENGISIAN, TINDAK BALAS, PEMENDAPAN, PENGELUARAN, dan REHAT dalam nisbah 2:11:1.5:1:0.5 untuk 16 jam satu kitaran. Sebuah reaktor kelompok berturutan digunakan sebagai reaktor kawalan (RC), manakala dua buah reaktor lain dioperasi dengan media pembawa yang berbeza, iaitu reaktor dengan kiub span poliuretana (RB1) dan reaktor dengan jejalur bulat polietilena (RB2). Setelah keadaan mantap tercapai dengan 'base mix' sebagai sumber karbon tunggal, PNP ditambah kepada reaktor-reaktor dengan kepekatan bertambah daripada 150 sehingga 350 mg/L selaras dengan kadar muatan PNP daripada 0.158 sehingga 0.368 kg/m³ d.

Didapati bahawa PNP telah disingkir lengkap untuk semua reaktor sehingga kadar muatan PNP mencapai 0.368 kg/m³ d. Peningkatan kadar muatan PNP telah menyebabkan penurunan kadar penguraian PNP. Reaktor SBBR menghasilkan kadar penyingkiran PNP yang lebih baik berbanding

dengan reaktor SBR. Diperhatikan bahawa penyingkiran AN hampir lengkap untuk semua reaktor sebelum PNP ditambahkan. Apabila kepekatan PNP yang ditambah telah meningkat kepada 350 mg/L, purata kecekapan penyingkiran AN bagi reaktor RC and RB2 masing-masing telah menurun kepada 86 dan 96%. Namum, reaktor RB1 masih mampu mencapai penyingkiran AN hampir 100%. Berdasar pada pencapaian keseluruhan reaktor-reaktor dan kajian kinetik, keberkesanan penyingkiran PNP dan AN dalam reaktor-reaktor telah disusun dalam turutan berikut: $RB1 > RB2 > RC$.

Dengan menggunakan analisis GC-MS, HPLC dan UV-Vis, mineralisasi lengkap PNP telah dipastikan tercapai dalam semua reaktor. Pengenalpastian laluan biopenguraian PNP melibatkan penyingkiran kumpulan nitro sebagai nitrit pada peringkat awal dan pembentukan hydroquinon sebagai salah satu perantaraan. Namum, perantaraan p-benzoquinon tidak dapat dikesan dalam proses penguraian PNP dalam kajian ini.

COMPARATIVE STUDY OF SEQUENCING BATCH BIOFILM REACTOR AND SEQUENCING BATCH REACTOR IN *p*-NITROPHENOL REMOVAL

ABSTRACT

The objective of this study are: (1) to compare the biodegradation of PNP under sequencing batch biofilm reactor (SBBR) and sequencing batch reactor (SBR) operations and (2) to identify the biodegradation pathway of *p*-nitrophenol (PNP) in mixed cultured activated sludge system. Three identical plexiglass reactors were operated with FILL, REACT, SETTLE, DRAW and IDLE periods in the ratio of 2:11:1.5:1:0.5 for a cycle time of 16 h. A SBR reactor (RC) was used as the control reactor, whereas the other two reactors were operated with different carrier media namely, reactor with polyurethane sponge cubes (RB1) and reactor with cylindrical-shaped polyethylene rings (RB2). After the steady state was attained with the base mix as the sole carbon source, the PNP was added to the reactors in increasing concentrations from 150 to 350 mg/L corresponding to the PNP loading rate from 0.158 to 0.368 kg/m³ d.

Complete removal of PNP was achieved for all reactors up to the PNP loading rate of 0.368 kg/m³ d. The increase of PNP loading rate had resulted in decreasing rates of PNP degradation. The SBBRs yielded better PNP removal rates compared to that of the SBR. It was observed that nearly complete AN removal was achieved for all reactors before the addition of PNP. When the influent PNP concentration was increased to 350 mg/L, the average

AN removal efficiency for reactors RC and RB2 were reduced to 86 and 96%, respectively. However, reactor RB1 still managed to achieve an almost 100% AN removal. Based on the general performances of the reactors and the kinetic studies, the performances of the reactors in PNP and AN removal were ranked in the following order: RB1 > RB2 > RC.

By using the GC-MS, HPLC and UV-Vis analyses, it was ascertained that complete mineralization of PNP was achieved in all the reactors. The pathway identified for the biodegradation of PNP involved the initial removal of the nitro group as nitrite and formation of hydroquinone as one of the intermediates.. However, the likely intermediate *p*-benzoquinone was not detected in the degradation process of PNP in this study.

CHAPTER 1 INTRODUCTION

1.0 Background

Over the years, the development of science and technology has experienced tremendous changes. The advancement in science and technology has improved the quality of life of human beings to a great extent. However, due to over-development and lack of environmental conservation measurements, a lot of environmental problems are becoming increasingly severe.

Today, many regions of the world are facing the problem of fresh water shortage as a result of population growth and continuing water pollution. As a result, how to protect and save the limited water resources for sustainable water uses have been the issues of increasing concern (Frederiksen, 1996). Installation of wastewater treatment systems to treat domestic sewage and industrial wastewater before they were discharged to the environment is one of the ways that help in preventing further contaminations of the limited water resources (Hidalgo et al., 2006).

Early wastewater treatment systems focus primarily on the removal of oxygen demanding materials such as organic matter and ammoniacal nitrogen that would deplete the dissolved oxygen (DO) in receiving waters. As industrialization and population growth continued, the problem of eutrophication is recognized (Manipura, 2007). The cause of eutrophication is mainly due to the discharge of wastewater containing excessive nutrients such as nitrogen and phosphorus into the aquatic environments like rivers, lakes and estuaries.

Thus, many studies during the last two decade have emphasized on the removal of nutrients in the wastewater (Munoz-Colunga and Gonzalez-Martinez, 1996; Helmer-Madhok et al., 2002).

Most recently, discharge of toxic organic chemicals (TOCs) to the environment has become the concern of many researchers. The challenge has been the need to remove TOCs to a low level, rather than just removing organic matter in general. Consequently, a lot of studies on the removal of TOCs in wastewater have been carried out over the last decade (Wobus et al., 1995; Ray et al., 1999; Karim and Gupta, 2003; Leven and Schnurer, 2005).

This research is primarily focused on the comparative study of sequencing batch biofilm reactor and sequencing batch reactor in *p*-nitrophenol (PNP) removal and to understand the effects of PNP in biological nitrogen removal. PNP was chosen as model TOC to be treated in this study because it is widely used in agricultural, pharmaceutical and dye industries as a synthetic intermediate in the manufacture of pesticides, medicines and dyes (Xing et al., 1999).

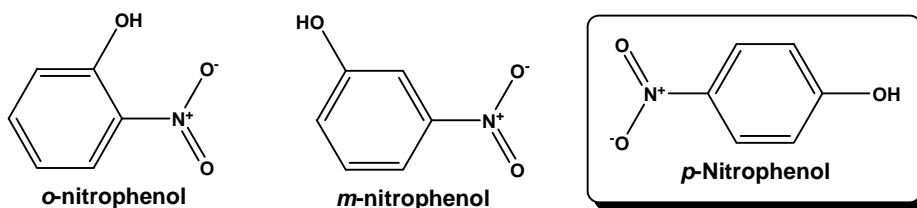
1.1 Characterization of *p*-Nitrophenol

p-Nitrophenol or 4-nitrophenol (abbreviated as PNP or 4NP) is a hazardous chemical substance widely used in industry. Removal of PNP from the wastewater is crucial as it is harmful to human health. It is important to recognize some of the basic characteristics of PNP before the removal methods are discussed. Some useful information such as the identity and physical-

chemical properties, the source and the fate of PNP as well as the effects of PNP on human health will be discussed.

1.1.1 Identity and Physical-Chemical Properties of PNP

p-Nitrophenol is a chemical compound that has a hydroxyl group and a nitro group that are attached to a benzene ring relatively in para position. The specific nomenclature of this substance is 1-hydroxy-4-nitrobenzene and it is more likely known as *p*-nitrophenol or 4-nitrophenol (Lide, 2002a). It has two constitutional isomers, namely, *o*-nitrophenol (2-nitrophenol) and *m*-nitrophenol (3-nitrophenol). They share the same empirical formula of $C_6H_5NO_3$. The structural formulas of the isomers are shown as follows:



PNP forms colorless to slightly yellow odorless crystals at room temperature with sweetish, then burning taste (O'neil et al., 2001). The physical and chemical properties of the substance are shown in Table 1.1.

Table 1.1: Physical-chemical properties of PNP.

Parameter	Value	Unit	References
Molecular mass	139.11	g/mol	Lide, 2002a
Melting point	113-114	°C	O'neil et al., 2001
Boiling point (decomposes)	279	°C	Weast, 1987
Vapour pressure (20 °C)	0.0003	mmHg	Leuenberger et al., 1985
Specific density (20 °C)	1.479	g/cm ³	Lide, 2002a
Water solubility (25 °C)	16.0	g/L	Leuenberger et al., 1985
pH value (5 g/L, H ₂ O, 24 °C)	4.4	-	Merck, 2007
Ignition temperature	510	°C	Merck, 2007

1.1.2 Sources of PNP

There is no known natural source of PNP in the environment. PNP is a xenobiotic organic chemical (XOC) produced by human beings. The term xenobiotic means “foreign to the biosphere”. It is produced either by the catalytic hydrolysis of *p*-nitrochlorobenzene or by the reaction of dilute HNO₃ with phenol followed by subsequent steam distillation to separate the *p*- isomer from the *o*- isomer (EPA, 1985; HSDB, 1989). From laboratory experiments, there is some evidence that PNP is formed in the air during the photochemical reaction of aromatic compounds such as benzene and toluene in the presence of nitric oxide, hydroxyl radicals or nitrous dioxide. PNP is also found in vehicular exhausts owing to the thermal reaction of fuel with oxides of nitrogen.

PNP is an important intermediate in the manufacturing of azo dyes and a number of pesticides such as parathion, fluorodifen, nitrofen and bifenox. It is also an intermediate in the synthesis of *p*-aminosalicylic acid (an antibiotics used to treat tuberculosis) and *p*-acetaminophenol which is commonly known as paracetamol (a common analgesic and antipyretic drug that is used for the relief of pains, headaches, and fever) (Bhatti et al., 2002). As a result, PNP is usually

detected in the wastewater of textile, pesticides and pharmaceutical manufacturing industries. Besides, the applications of pesticides will lead to the released of PNP into surface water as a result of runoff from agricultural lands as some of the pesticides especially parathion undergo degradation and hydrolysis to produce PNP (Bhatti et al., 2002). In addition, PNP is also found in the effluent of explosive production industry (Xing et al, 1999).

1.1.3 Fate of PNP

Photolysis and biodegradation are the major fate processes of PNP in water. Photolysis plays an important role for the degradation of PNP in near-surface water where attenuation of sunlight is normally minimal. The experimental half-life of the PNP biodegradation may be about 3.2 days in fresh water (Vaishnav and Korthals, 1988) and may range between 13 and 21 days in sea water (Van Veld and Spain, 1983). The half-life of PNP in top-soil may be about 1 day under aerobic conditions and 14 days under anaerobic conditions. The half-life of PNP in sub-soils may be about 40 days under aerobic conditions and is expected slower than that under anaerobic conditions (Loecke, 1985). It is believed that biodegradation may be the most important fate process for the degradation of PNP in soils. Information about the half-life of PNP in the air is not available yet. However, both photolysis and physical removal processes like gravitational settling of aerosols and wet deposition by rain and snow will probably be the fate processes of PNP in the air.

1.1.4 Effects of PNP on Human Health

There are no studies that directly investigate the effects of PNP on human beings. The information regarding the health effects caused by PNP comes from the studies in animals. The effects of PNP on human health are discussed following the routes of exposure such as inhalation, oral and dermal exposures.

According to Smith et al. (1988), rats exposed to dust atmospheres of PNP (sodium salt) at a concentration of 2119 mg PNP/m³, 6 hours/day for 10 days showed a decrease in absolute and relative lung weights after a 14 day recovery period. Besides, rats exposed to 112 mg of PNP/m³ as PNP sodium salt for 2 weeks showed a blood disorder which reduces the ability of the blood to carry oxygen to tissues and organs. It was suggested that, chemicals like PNP cause a similar blood disorder in humans. Thus, humans exposed for weeks or longer to high levels of PNP may develop the same types of blood disorders that animals do.

Vernot et al. (1977) reported that oral LD₅₀ values in mice and in rats were 470 mg/kg and 620 mg/kg, respectively, after gavage administration of PNP in corn oil. According to Plasterer et al. (1985), daily gavage doses of 400 mg of PNP/kg administered to pregnant mice during gestation days 7-15 had caused 19% lethality. Besides, early mortality was observed in rats after gavage administration of 70 mg PNP/kg in water for 13 weeks. The clinical observations prior to death included prostration, wheezing and dyspnea (Hazleton Lab, 1989).

Application of PNP on the skin of rats with daily doses of 50-250 mg PNP/kg for 120 days resulted in dose-related dermal irritation. The types of irritation consist of erythema, scabbing, scaling and cracking of the skin (Angerhofer, 1985). However, no treatment related deaths were observed in rats following the dermal exposure to PNP with the doses and period mentioned above.

1.2 Removal of PNP

Removal of PNP in wastewater can be achieved by physical, chemical or biological treatment processes and a variety of combinations of the treatment processes. Among the major physical treatment processes for PNP removal are adsorption (Sayilkan et al., 2004; Tewari and Boodhoo, 2005), ultrasonic irradiation (Sivakumar et al., 2002) and microwave assisted oxidation (Bo et al., 2006).

The application of chemical treatment process for PNP removal is limited and only electrochemical oxidation (Yuan et al., 2006) has been recognized so far. However, the combinations of physical and chemical treatment processes are much popular. They include photo-catalytic degradation of PNP with ultraviolet (UV) and titanium dioxide (TiO_2) (Wang et al., 1998), photo-electrocatalytic degradation of PNP using Ti/TiO₂ thin-film electrode (Jorge et al., 2005), activated carbon adsorption integrated with electrocatalysis (Zhou and Lei, 2006) and photooxidative degradation of PNP in UV/H₂O₂ (Daneshvar et al., 2007).

Biological treatment processes are the most widely used methods in PNP removal and can be classified as aerobic suspended growth (Tomei et al., 2003), aerobic attached growth (Ray et al., 1999; Alber et al., 2000), combined aerobic suspended and attached growth (Xing et al., 1999; Bhatti et al., 2002), anaerobic suspended growth (Donlon et al., 1996; Karim and Gupta, 2001; Karim and Gupta, 2003; Kuscu and Sponza, 2005), combined anaerobic/aerobic suspended growth (Liu, 2000; Sponza and Kuscu, 2005; Buitron et al., 2005; Kuscu and Sponza, 2006) and combined anaerobic/aerobic attached growth (Melgoza & Buitron, 2001) processes.

1.2.1 Physical Treatment Process

Sayilkan et al. (2004) used pyrophyllite as an adsorbent to remove PNP from aqueous solutions. The raw pyrophyllite mineral was treated with prehydrolyzed N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (APEO) coupling agent to change its naturally hydrophobic surface into hydrophilic surface. The adsorption capacity was found to be 0.268 mg/g for modified pyrophyllite compared to 0.105 mg/g for untreated pyrophyllite. However, the percentage of PNP removal was not mentioned in the study.

Removal of PNP from aqueous solutions through adsorption on antimony, cadmium and zirconium ferrocyanides (SbFc, CdFc & ZrFc) was suggested by Tewari and Boodhoo (2005). The adsorption capacity for SbFc, CdFc and ZrFc were found to be 260.00, 290.00 and 166.67 mg/g, respectively. The study also reported that the most effective pH for PNP removal was found to be 6.0. However, the percentage of PNP removal was not shown in this study as well.

A treatment process that utilized ultrasonic irradiation to induce sonochemical degradation of PNP in an aqueous solution was adopted by Sivakumar et al. (2002). The performances of the degradation of PNP under three operating frequencies (25, 40 and 25+40 kHz) have been studied. It was reported that, the rate of degradation under the effect of dual frequency mode (25+40 kHz) was greater than that under single frequency modes. However, the PNP removal efficiency of this process needs to be improved as the highest percentage of PNP removal that can be achieved under optimum conditions was 11.36 % only.

1.2.2 Chemical Treatment Process

The treatment of PNP by cathode reduction and electro-Fenton methods were carried out by Yuan et al. (2006). The results showed that the degradation of PNP was much faster in the cathode cell than in the anodic cell. Besides, the degradation of PNP was significantly enhanced by the addition of aeration and Fenton's reagent. More than 98% removal of PNP and about 13% removal of total organic carbon were reported. The disadvantage of this process is that nitrite released from the degradation of PNP was not removed.

1.2.3 Physical-Chemical Treatment Process

The photo-electrochemical degradation of PNP using titanium dioxide thin-film electrode was studied by Jorge et al. (2005). The study reported that the optimum conditions for the degradation of PNP were found to be in perchlorate medium at pH 2 with +1.0 V of applied potential on Ti/TiO₂ over 3 h

of experiment. This treatment process was found to be not efficient in PNP removal as the percentage of PNP removal was about 60% only.

Daneshvar et al. (2007) investigated an integrated process consisting of the application of UV light and H_2O_2 for the photo-oxidative degradation of PNP in an aqueous solution. The study showed that complete degradation of PNP could be achieved in 13 min. However, the removal efficiency was a function of light intensity, H_2O_2 and PNP concentrations, where high H_2O_2 doses or UV intensity were required for the degradation of high concentration of PNP in the solution.

An integrated process involving electrocatalysis and activated carbon (AC) adsorption for the removal of PNP was adopted by Zhou and Lei (2006). It was found that the PNP of initial concentration 150 mg/L could be almost completely removed within 30 min. However, the drawback of this process is the requirement of the addition of pure oxygen gas which indirectly increases the cost of the treatment.

1.2.4 Biological Treatment Process

Over the years, many toxic organic compounds have proven to be treatable using biological treatment process. (Herzbrun et al., 1985; Brenner et al., 1992; Lee and Lim, 2003). From the literature survey, it was found that biological treatment system had been the most broadly used method in the removal of PNP compared to physical or chemical treatment systems. The biological treatment process could basically be classified as either an aerobic or

anaerobic process. For example, the processes in the traditional activated sludge (AS), sequencing batch reactor (SBR), aerated solid-bed reactor and soil perfusion bioreactor systems follow an aerobic path whereas those in upflow anaerobic sludge blanket (UASB) reactor and anaerobic baffled reactor systems follow an anaerobic path. However, some processes involving a combination of anaerobic and aerobic paths such as anaerobic migrating blanket reactor (AMBR)/aerobic completely stirred tank reactor (CSTR), anaerobic/aerobic SBR systems have also been suggested recently.

The biological treatment processes have some advantages and disadvantages in comparison to physical and chemical treatment processes. For example, biological treatment processes are more efficient and cost effective than physical and chemical treatment processes when the concentrations of reacting constituents in the wastewater are low. In biological operations, the soluble pollutants are converted either into an innocuous form, such as carbon dioxide or nitrogen gas, or into new biomass, which can be easily removed by a physical operation. Hence, no toxic wastes are generated from biological treatment processes. Besides, some studies have shown that sludges from biological treatment processes are useful and can be recycled as fertilizer, building material and bio-fuel (Fujita et al., 2005; Balasubramanian et al., 2006; Dewil et al., 2007). However, biological treatment processes also have some disadvantages compared to the physical and chemical ones. For instance, the transformation rates of insoluble inorganic matter by microorganisms are too low to be of practical importance. Thus, insoluble inorganic matter is typically removed by preliminary physical unit operations for

further treatment and disposal. Besides, biological treatment processes are not efficient for treating wastewater containing high concentration of chemical substances of high toxicity.

1.3 The Activated Sludge Process

Over the decade, activated sludge process has gained its popularity in the treatment of toxic chemical compounds. The activated sludge process was first introduced by Arden and Lockett in England in 1914. It still remains as the most widely used biological wastewater treatment process today. As discussed in Section 1.2.4, activated sludge is an aerobic suspended growth process that uses microorganisms to remove soluble organic matters. Over the years, activated sludge process has evolved into many kinds of wastewater treatment systems such as conventional, complete-mix, step aeration, pure oxygen, sequencing batch reactor, contact stabilization, extended aeration, oxidation ditch, deep tank and deep shaft systems (Tchobanoglous and Burton, 1991).

1.3.1 Sequencing Batch Reactor (SBR)

Among the activated sludge system, SBR system is gaining increasing popularity in terms of lab-scale scientific studies to full-scale applications for domestic and industrial wastewater treatment plants. The advantages of SBR system (Irvine and Ketchum, 1989; Wobus et al., 1995; Louzeiro et al., 2002) are as follows:

- Simple and cost effective;
- Ability to combine aerobic and anoxic phases in a single reactor;

- High degree of process flexibility in terms of sequence and cycle time;
- Near ideal quiescent settling conditions;
- More resistant to fluctuating influent loading.

A SBR is an activated sludge system running on the fill and draw principle. In the SBR operation, the aeration and sedimentation processes are carried out sequentially in the same tank rather than simultaneously in separate tanks as for the conventional activated sludge system (Arora et al., 1985). The operation of the SBR system is commonly carried out sequentially in five phases namely, FILL, REACT, SETTLE, DRAW and IDLE, respectively.

The removal of PNP using SBR system was studied by Tomei et al. (2004). It was reported that both long feed phase and high biomass concentration were effective in reducing the substrate concentration peak and then improving the process efficiency. However, the nitrite released from the degradation of PNP was not treated.

1.3.2 Sequencing Batch Biofilm Reactor (SBBR)

A SBBR is a combined suspended and attached growth (CSAG) system that consists of a SBR added with attached growth media. The accumulation of biomass on the carrier media in SBBR provides an additional biomass inventory. The high biomass concentrations that can be achieved by a SBBR system result in shorter HRT in comparison to ordinary SBR system with equivalent solid retention time (SRT) (Lessel, 1994). The shorter HRT results in smaller reactor size and greater treatment capacity at the same reactor size.

Studies also suggested that attached growth system should be more resistant to inhibitory effects of XOCs than suspended growth system (Stevens, 1988; Gantzer, 1989). This is because the inner layers in a biofilm experience a lower substrate concentration than the outer layers, which gives a higher reaction rate for an inhibitory substrate. Thus, the overall reaction rate will be higher than the bulk substrate concentration would suggest (Grady et al., 1999).

Biological treatment of leachates from hazardous waste landfills using SBBR system was adopted by Dollerer and Wilderer (1996). It was reported that in the case of organic compounds with a low bioavailability, when specialized organisms are required or when a decrease in the BOD/COD ratio of wastewater is experienced, a SBBR system is preferable.

Over the years, most of the SBBR systems were studied for their advantages in biological nutrients removal (Garzon-Zuniga and Gonzalez-Martinez, 1996; Munoz-Colunga and Gonzalez-Martinez, 1996). However, studies on the application of SBBR in the treatment of XOCs were relatively limited. Thus, this research employs SBBR systems to treat PNP so that further information regarding the treatment of XOC in SBBR can be obtained.

1.4 Biological Nitrogen Removal

Removal of nitrogen in wastewater prior to disposal has drawn much attention from the researchers (Fang et al., 1993; Halling-Sorensen & Jorgensen, 1993; Tsai et al., 2003; Pambrun et al., 2004) over the decades as the presence of nitrogen in the secondary-treated wastewater still threatens the

quality of receiving waters. From the literature survey, it has been shown that biological processes especially the activated sludge systems are reliable and cost-effective in the removal of nitrogen (Randal, 1992; Munch et al., 1996; Louzeiro et al., 2002). Conventionally, biological nitrogen removal is achieved through two principal processes namely, assimilation and nitrification-denitrification. However, some researchers have adopted a new approach known as nitritation-denitritation processes as a replacement for the nitrification-denitrification processes in recent years (Pollice et al., 2002; Lai et al., 2004; Fux et al., 2006).

1.4.1 Assimilation

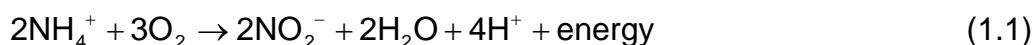
Microorganisms in the biological treatment process required nitrogen as a nutrient for continuous growth. The term assimilation can be defined as a process which transforms ammoniacal nitrogen in the wastewater into the cell mass of the microorganisms. On the other hand, a portion of this ammoniacal nitrogen will be returned to the wastewater on the death and lysis of the cells which is known as ammonification (Grady et al., 1999).

1.4.2 Nitrification and Denitrification

There are two steps involved in the removal of nitrogen in the wastewater through nitrification-denitrification process. The first step is the nitrification process that needs to be carried out in aerobic condition and the second step is the denitrification process that needs to be operated under anoxic condition.

The nitrification process involves the oxidation of ammoniacal nitrogen (AN) to nitrite nitrogen (NO_2^- -N) mediated by *Nitrosomonas* microorganisms and the subsequent oxidation of nitrite nitrogen to nitrate nitrogen (NO_3^- -N) mediated by *Nitrobacter* (Hu et al., 2004). *Nitrosomonas* and *Nitrobacter* are autotrophic bacteria that obtain their energy from the oxidation of reduced nitrogen and use carbon dioxide as the carbon source for the synthesis of their cellular material (Tchobanoglous and Burton, 1991).

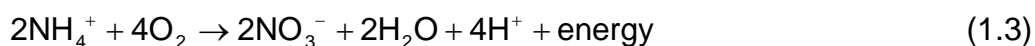
The reaction for the oxidation of ammonium ions by *Nitrosomonas*:



The reaction for the oxidation of nitrite ions by *Nitrobacter*:



The overall nitrification reaction:



The population size of nitrifying bacteria within the activated sludge is very small, typically 2-5% in comparison to the population size of heterotrophs as the growth rate of nitrifying bacteria is slow compared to the heterotrophic bacteria (Randall, 1992). Among the nitrifying bacteria, the population size of *Nitrosomonas* is larger than that of *Nitrobacter* as *Nitrobacter* obtain less energy from the oxidation of nitrite than what is obtained from the oxidation of ammonium ions by *Nitrosomonas*. Besides, *Nitrosomonas* has a shorter generation time and is able to increase quickly in the number as compared to

Nitrobacter. The difference in generation time between *Nitrosomonas* and *Nitrobacter* is the key factor for the buildup of nitrite ions during adverse operational conditions such as inadequate dissolved oxygen, hydraulic washout, toxicity and so on (Lim, 2006).

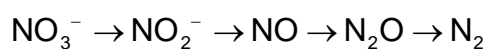
Studies have shown that nitrification, being a biological process involving activated sludge, is affected by many process variables including pH and temperature (Painter and Loveless, 1983), dissolved oxygen (Munch et al., 1996; Park and Noguera, 2004), ammonia and nitrite concentrations (Carrera et al., 2004), exogenous carbon source (Tam et al., 1992), chemical oxygen demand (COD)/total Kjeldahl nitrogen (TKN) ratio as well as HRT and SRT (Pollice et al., 2002; Sharma and Gupta, 2004). Some of the optimum conditions for the operation of nitrification suggested by various researchers are shown in Table 1.2.

Table 1.2: Optimum conditions for nitrification.

Variables	Conditions	References
pH	7.5-8.5	Painter and Loveless, 1983
Temperature	24-35 °C	Younos, 1987
DO	>1 mg/L	Tchobanoglous and Burton, 1991
SRT	8-20 d	Tchobanoglous and Burton, 1991

In addition, effects of toxic pollutants such as heavy metals and xenobiotic organic chemicals on the bioactivity of activated sludge in nitrogen removal were also reported by various researchers (Hu et al., 2002; Juliastuti et al., 2003; Gheewala et al., 2004; Ong et al., 2005).

It should be noted that the oxidation of AN to NO_2^- -N or NO_3^- -N does not remove the nitrogen in the system but is merely a change of oxidation state for the nitrogen species. Nitrite or nitrate nitrogen, the products of the nitrification process, need to be reduced to gaseous nitrogen before they can be released to the atmosphere to achieve the objective of nitrogen removal for the treatment system. This process is known as denitrification and it removes nitrogen from the system in the following sequence:



Conversion of nitrate to nitrite is the first step in the denitrification process. It is followed by further reduction of nitrite to gaseous compounds such as nitric oxide, nitrous oxide and finally the nitrogen gas (Tchobanoglous and Burton, 1991).

As described above, denitrification can only proceed under anoxic conditions with the DO concentration lower than 2% saturation (Kiff, 1972), and when additional carbon source is available to act as an electron donor. The denitrification process can be accomplished by a wide range of facultative anaerobes such as *Achromobacter*, *Alcaligenes*, *Denitrobacillus*, *Micrococcus*, *Pseudomonas*, *Spirillum* and *Xanthomonas* (Painter, 1970; Tiedje, 1988).

The rate of denitrification is affected by pH, temperature, as well as NO_2^- -N, NO_3^- -N and external carbon source concentrations. The optimum pH lies between 7.0 and 8.0 while the optimum temperature is 25-30 °C. It is

also learned that alkalinity is generated as denitrification proceeds resulting in an increase in pH (Gray, 2004).

1.4.3 Nitritation and Denitrification

Nitritation process is basically identical to nitrification process. The only difference is that the step for the oxidation of nitrite to nitrate in nitrification process is omitted in nitritation process. Conversely, denitrification is a process which directly removes nitrite instead of nitrate in the removal of nitrogen. In order to prevent nitrite oxidation, aerobic and anoxic periods were alternated at pre-set time intervals (Fux et al., 2006). It was reported that the prevention of nitrite oxidation saves the oxygen required for the nitrification process (up to 25% total oxygen demand) and 40% of the organic carbon required in denitrification process (Turk and Mavinic 1989; Hellinga et al., 1998). Figure 1.1 shows the processes involved in the removal of nitrogen species.

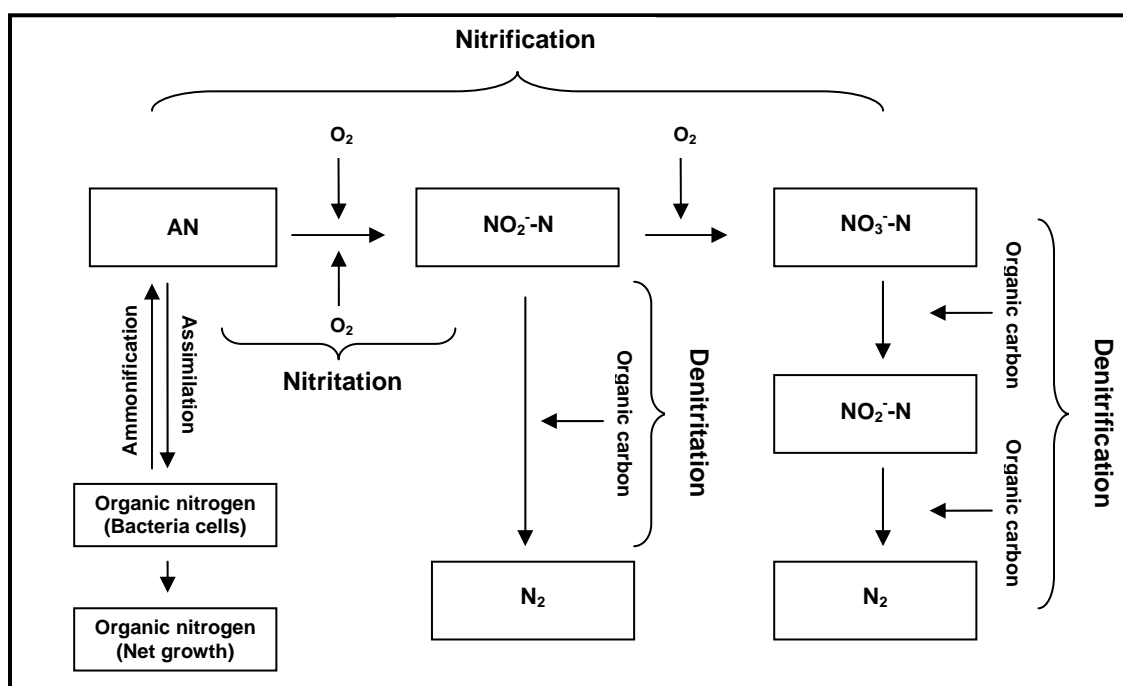


Fig. 1.1: Removal of nitrogen species.

1.5 Kinetic Modeling

In Section 1.2.4 many types of biological treatment processes were discussed. Understanding how each performs would be a difficult task if each were to be treated as a unique entity. As different systems may have different designs and configurations, different configurations will lead to different outcomes. Therefore, it is not able to evaluate the performance of two reactors based on the outcomes unless they are identical in design and configuration. Hence, kinetic study is essential in comparing the performance of different systems because kinetics is concerned with the rates at which reactions take place regardless of the final outcome. Kinetic modeling is the application of mathematical models to analyze the performance of the reactors, thus providing the stage for understanding how different configurations of a system will perform differently. In this research, kinetic study has been carried out to investigate the influence of PNP loading on the removal of PNP and nitrogen species.

1.5.1 Removal of PNP

Kinetic modeling for the removal of toxic organic compounds in activated sludge system had been studied by many researchers (Carvalho et al., 2001; Tomei et al., 2004; Nuhoglu and Yalcin, 2005; Sahinkaya and Dilek, 2005). It has been shown that high substrate concentrations would inhibit the growth and may distorted the metabolism of microorganisms (Edwards, 1970). PNP is one of the toxic organic compounds that impose inhibitory effects on the metabolism of the microorganisms in the activated sludge system.

Conventionally, the Monod equation is used to describe the kinetics of microbial growth on organic substrates in wastewater for the understanding of the effects of increasing substrate concentrations to the microorganisms. However, with the presence of inhibitory organic substance, the specific growth rate of microorganisms after reaching maximum will then decline as the inhibitory substrate concentration is increased. The Monod equation is not adequate for depicting this situation. Therefore, another model known as the Haldane equation is required as it contains an additional parameter, K_i , the inhibition coefficient which can indicate the inhibitory effects of the inhibitory substrate towards the microorganisms. The model proposed by Haldane was as follows:

$$-\frac{dS}{dt} = \frac{V_{\max} S}{(K_s + S) + (1 + \frac{S}{K_i})} \quad (1.4)$$

where,

$$-\frac{dS}{dt} = \text{Substrate removal rate}$$

$$S = \text{Substrate concentration (mg/L)}$$

$$V_{\max} = \text{Maximum removal rate (mg/L min)}$$

$$K_s = \text{Half saturation constant (mg/L)}$$

$$K_i = \text{Inhibition constant (mg/L)}$$

1.5.2 Removal of AN

The removal of AN was identified to involve two stages. It was observed that the first stage of AN removal was mainly due to simultaneous nitrification

and denitrification (SND) processes as the production of oxidized nitrogen was not observed (Lim, 2005). The second stage of AN removal was followed by the production of oxidized nitrogen. This suggests autotrophic nitrification in the second stage of AN removal. In this study, interest was put on the rate of AN removal in the second stage, which is assumed to follow the first-order kinetics as follows:

$$[AN]_t = [AN]_0 \exp(-k_{AN} \cdot t) \quad (1.5)$$

where,

$[AN]_t$ = Concentration of AN at time t

$[AN]_0$ = Initial concentration of AN

k_{AN} = Pseudo first-order rate constant for the removal of AN

t = Time of reaction

1.6 Degradation Pathways of PNP

Over the years, many studies on PNP removal in wastewater have been carried out. Among others, many researchers were interested to find out how PNP was removed in the treatment processes (Spain and Gibson 1991; Jain et al., 1994; Yuan et al., 2006; Daneshvar et al., 2007). These efforts were aided by emerging new and advanced instruments, thus enabling better detection and identification of the organic compounds. To date, the degradation pathways of PNP that have been identified can be classified into two categories. One is the degradation pathway of PNP in non-biological processes and the other one is the biodegradation pathways of PNP through biological processes.

1.6.1 Degradation of PNP in Physical-chemical Process.

Yuan et al. (2006) have investigated the treatment of PNP by cathode reduction and electro-Fenton methods. It was proposed that PNP was first reduced on cathode to generate *p*-nitrosophenol, which is further reduced to *p*-aminophenol. Then, *p*-aminophenol is oxidized to hydroquinone and *p*-benzoquinone by hydroxyl radicals. The intermediates were further oxidized to ring opening compounds.

On the other hand, the degradation of PNP in UV/H₂O₂ process was studied by Daneshvar et al. (2007). It was reported that the degradation of PNP started with the reaction of hydroxyl radicals with the aromatic groups to form *p*-nitrocatechol. Then, *p*-nitrocatechol will further react with hydroxyl radical to form 1,2,4-benzenetriol and nitrite was released in the meantime. Further reaction of the 1,2,4-benzenetriol with hydroxyl radicals led to ring opening and the formation of oxygenated aliphatic compounds and eventually achieved complete mineralization of the PNP.

It was also reported that direct attack of the hydroxyl radicals to nitro-group position led to the formation of hydroquinone. Similarly, hydroquinone further reacted with hydroxyl radicals to form 1,2,4-benzenetriol and 1,2,4-benzenetriol was further degraded following the pathway described above.

1.6.2 Biodegradation of PNP in Biological Process

Biodegradation of PNP by *Moraxella* sp. was studied by Spain and Gibson (1991). The results indicated that the pathway for the biodegradation of

PNP involved the initial removal of the nitro group as nitrite and formation of hydroquinone. According to Spain and Gibson (1991), the initial replacement of nitro group by hydroxyl group by the *Moraxella* sp. had been suggested by Simpson and Evans (1953) for a *Pseudomonas* sp. grown on PNP. However, this contrasted with the initial conversion of PNP to *p*-nitrocatechol suggested by Raymond and Alexander (1971) for a *Flavobacterium* sp.. The hydroquinone was further converted to γ -hydroxymuconic semialdehyde. Then, γ -hydroxymuconic semialdehyde was converted to maleylacetic acid and eventually to β -ketoadipic acid.

An alternative pathway for the biodegradation of PNP by an *Arthrobacter* sp. was suggested by Jain et al. (1994). The results showed that the initial reaction in the catabolism of PNP by *Arthrobacter* sp. was a monooxygenase-catalyzed hydroxylation of the ring at either the *ortho* or the *meta* position. The initial product was either *p*-nitrocatechol or *p*-nitroresorcinol. The *p*-nitrocatechol and *p*-nitroresorcinol were converted to 1,2,4-benzenetriol with the released of nitro group as nitrite. The 1,2,4-benzenetriol then underwent *ortho* ring fission to form maleylacetic acid which further degraded to β -ketoadipic acid. The pathways for the biodegradation of PNP proposed by Spain and Gibson (1991) and Jain et al. (1994) are shown in Fig. 1.2.