MOVING BED SEQUENCING BATCH REACTOR: OPTIMIZATION OF OPERATIONAL PARAMETERS FOR NITROGEN AND 4-CHLOROPHENOL REMOVAL

by

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LIST OF ABBREVIATIONS

4-CP	4-Chlorophenol
AOB	Ammonium oxidizing bacteria
ATU	Allythiourea
CA	Continuous aeration
COD	Chemical oxygen demand
DO	Dissolved oxygen
IA	Intermittent aeration
IA-MBSBR	Intermittent aeration-moving bed sequencing batch reactor
MBSBR	Moving bed sequencing batch reactor
MLSS	Mixed liquor suspended solids
NH_4^+-N	Ammonium-nitrogen
NO_2^--N	Nitrite-nitrogen
NO_3^--N	Nitrate-nitrogen
NOB	Nitrite oxidizing bacteria
PU	Polyurethane
SBR	Sequencing batch reactor
SEM	Scanning electron microscope
SND	Simultaneous nitrification and denitrification
SS	Suspended solids
SVI	Sludge volume index
TN	Total nitrogen

LIST OF NOMENCLATURE

d	Average diameter of the pore of PU foam
$k_{4\text{-}CP}$	Pseudo-zeroth-order rate constant of 4-CP removal
k_{AN}	Pseudo-zeroth-order rate constant of NH_4^+-N removal
K_i	Inhibition constant
K_s	Half-saturation constant
$R_{S,\,max}$	Maximum specific degradation rate
S_i	Each pore surface area of PU foam
V_i	Each pore volume of PU foam

REAKTOR KELOMPOK BERTURUTAN PELANTAR BERGERAK: PENGOPTIMUMAN PARAMETER BEROPERASI BAGI PENYINGKIRAN NITROGEN DAN 4-KLOROFENOL

ABSTRAK

Reaktor kelompok berturutan pelantar bergerak (MBSBR) yang menggabungkan biojisim pertumbuhan-terampai dan pertumbuhan-tertambat dalam reaktor tunggal telah digunakan secara meluas di logi pengolahan air buangan. Namun, maklumat tentang pengolahan nitrogen yang terkandung dalam air buangan melalui MBSBR masih lagi tidak banyak didapati. Oleh itu, lebih banyak perhatian daripada penyelidik untuk mengkaji potensi penggunaan MBSBR dalam penyingkiran nitrogen secara biologi adalah diperlukan. Penyelidikan ini bertujuan untuk mengoptimum penggunaan kiub busa poliuretana (PU) sebagai media penyokong dan mengaplikasi strategi pengudaraan optimum semasa fasa TINDAK BALAS dalam MBSBR untuk meningkatkan penyingkiran nitrogen dalam air buangan yang mengandungi nisbah rendah COD/N. Tambahan lagi, kesan 4-klorofenol (4-CP) terhadap penyingkiran 4-CP dan nitrogen secara serentak dalam MBSBR juga dikaji.

Pada awalnya, MBSBR dipadatkan dengan pelbagai saiz kiub busa PU dengan tujuan untuk mengkaji kesan saiz media penyokong terhadap penyingkiran nitrogen. Seterusnya, strategi pengudaraan semasa fasa TINDAK BALAS diubah daripada pengudaraan berterusan kepada berselang-seli dan prestasi kedua-dua strategi pengudaraan dalam menyingkirkan nitrogen dibandingkan. Biojisim pertumbuhan-tertambat daripada kiub busa PU juga dikeringkan dan kemudiannya dimasukkan dalam MBSBR dengan tujuan mengkaji kemungkinan untuk digunakan sebagai sumber karbon pepejal alternatif dalam meningkatkan penyingkiran nitrogen. Dalam kehadiran 4-CP, strategi pengudaraan semasa fasa TINDAK BALAS diubah

secara bertahap-tahap daripada pengudaraan berselang-seli kepada berterusan dengan kajian dalam penyingkiran 4-CP dan nitrogen secara serentak dijalankan pada masa yang sama semasa setiap tahap pengubahan. Akhir sekali, semasa 4-CP ditambah ke dalam MBSBR dengan kepekatan yang semakin meningkat, kesan saiz kiub busa PU terhadap penyingkiran serentak 4-CP dan nitrogen juga dikaji.

Penggunaan kiub busa PU sebagai media penyokong dalam MBSBR mampu menjana karbon tersimpan setelah kiub tersebut ditumbuhi dengan biojisim pertumbuhan-tertambat yang mana hasilnya memudahkan penyingkiran nitrogen melalui proses nitrifikasi dan denitrifikasi serentak (SND). Disebabkan oleh kuantiti biojisim pertumbuhan-tertambat meningkat dengan pensusutan saiz kiub busa PU, MBSBR yang dipadati dengan saiz kiub busa PU yang terkecil, iaitu kiub 8-mL, mencapai peratusan penyingkiran total nitrogen (TN) yang tertinggi (37.3%). Peningkatan selanjutnya ke tahap 57.0% penyingkiran TN juga diperhatikan apabila strategi pengudaraan diubah daripada pengudaraan berterusan kepada berturutan tempoh 1 jam pengudaraan diikuti dengan tempoh 1 jam tanpa pengudaraan dalam MBSBR yang mana ditafsirkan sebagai strategi 1:1 pengudaraan berselang-seli (IA). Ini disebabkan oleh pengaplikasian strategi pengudaraan berselang-seli yang mana lebih banyak karbon tersimpan dapat diperlihara jika dibandingkan dengan strategi pengudaraan berterusan. Selain itu, dengan strategi 1:1 IA, penggunaan kiub busa PU yang diselaputi-biojisim kering yang boleh digunakan semula didapati mampu meningkatkan penyingkiran TN ke tahap 75.0%. Dengan mengubah pengudaraan berselang-seli kepada strategi 1:2 IA, sedikit peningkatan penyingkiran TN ke tahap 79.0% dapat dikesan. Walaupun bagaimanapun, penggunaan strategi 1:3 IA telah menyebabkan penyingkiran ammonium-nitrogen (NH_4^+-N) menjadi tidak lengkap dalam MBSBR.

Dalam kehadiran 4-CP pada kepekatan 50 mg/L, kadar penyingkiran 4-CP dan NH_4^+-N diperhatikan meningkat secara beransur-ansur apabila semakin banyak tempoh tanpa pengudaraan diubah secara bertahap-tahap kepada tempoh pengudaraan menunjukkan bahawa strategi pengudaraan berterusan adalah lebih sesuai digunakan untuk penyingkiran 4-CP dan nitrogen secara serentak dalam MBSBR. Walaupun demikian, prestasi MBSBR dalam penyingkiran serentak 4-CP dan nitrogen merosot dengan peningkatan kepekatan 4-CP. Pada kepekatan 4-CP yang melebihi 300 mg/L, MBSBR yang dipadati dengan kiub busa PU 27-mL didapati berprestasi lebih baik daripada MBSBR yang lain. Ini disebabkan oleh biojisim pertumbuhan-tertambat yang berada di dalam kiub 27-mL adalah lebih terlindung daripada kesan perencatan 4-CP jika dibandingkan dengan kiub yang lebih kecil dan mobiliti kiub 27-mL adalah lebih cepat jika dibandingkan dengan kiub yang lebih besar. Oleh itu, dalam pengolahan 4-CP yang berkepekatan tinggi, MBSBR yang dipadati dengan kiub busa PU 27-mL merupakan sistem yang terpilih bagi penyingkiran 4-CP dan nitrogen secara serentak.

MOVING BED SEQUENCING BATCH REACTOR: OPTIMIZATION OF OPERATIONAL PARAMETERS FOR NITROGEN AND 4-CHLOROPHENOL REMOVAL

ABSTRACT

Moving bed sequencing batch reactor (MBSBR) which incorporates both suspended-growth and attached-growth biomasses in a single reactor has been widely used in wastewater treatment plants. Nevertheless, information on the treatment of nitrogen containing wastewater via MBSBR is still scarce. Thus, more attention from researchers to explore the potential of using MBSBR in biological nitrogen removal is needed. This study aimed to optimize the use of polyurethane (PU) foam cubes as a support media and to apply the optimum aeration strategy during the REACT phase in the MBSBR for the enhancement of nitrogen removal in low COD/N ratio wastewater. In addition, the effect of 4-chlorophenol (4-CP) on the simultaneous 4-CP and nitrogen removal in the MBSBR was also investigated.

The MBSBR was initially packed with PU foam cubes of various sizes to determine the effect of support media size on nitrogen removal. Subsequently, the aeration strategy during the REACT phase was converted from continuous to intermittent aeration and the performance of both aeration strategies in removing nitrogen was compared. The attached-growth biomass from the PU foam cubes was also dried and later added into the MBSBR to assess the feasibility to be utilized as an alternative solid carbon source in enhancing the nitrogen removal. In the presence of 4-CP, the aeration strategy during the REACT phase was progressively converted from intermittent to continuous aeration with the investigation of simultaneous 4-CP and nitrogen removal being conducted at the same time during each conversion. Finally, when the MBSBR was spiked with increasing concentrations of 4-CP, the

effect of PU foam cubes size on the simultaneous removal of 4-CP and nitrogen was also evaluated.

The use of PU foam cubes as a support media in the MBSBR could generate carbon storage once the attached-growth biomass grew on and into the cubes which eventually facilitated the removal of nitrogen via the simultaneous nitrification and denitrification (SND) process. Since the quantity of attached-growth biomass formed increased with the decrease of PU foam cubes sizes, the MBSBR packed with the smallest size of PU foam cubes, namely 8-mL cubes, achieved the highest percentage of total nitrogen (TN) removal (37.3%). Further increase of TN removal to 57.0% was also observed when the aeration strategy was converted from continuous aeration to consecutive 1 h of aeration period followed by 1 h of non-aeration period in the MBSBR which was defined as the 1:1 intermittent aeration (IA) strategy. This was due to the application of intermittent aeration strategy which could retain more carbon storage as compared to the continuous aeration strategy. Furthermore, with the 1:1 IA strategy, the use of recyclable dried biomass-coated PU foam cubes was found to enhance the TN removal to 75.0%. By varying the intermittent aeration to 1:2 IA strategy, a slight increase of TN removal to 79.0% was detected. However, the use of 1:3 IA strategy had caused incomplete removal of ammonium-nitrogen (NH_4^+-N) in the MBSBR.

In the presence of 50 mg/L of 4-CP, the removal rates of 4-CP and NH_4^+-N were observed to increase gradually when more of the non-aeration periods were progressively converted to the aeration periods indicating that continuous aeration is the preferred strategy for simultaneous 4-CP and nitrogen removal in the MBSBR. Nevertheless, the performance of MBSBR on the simultaneous removal of 4-CP and nitrogen deteriorated with increasing 4-CP concentrations. At the 4-CP concentrations higher than 300 mg/L, the MBSBR packed with 27-mL PU foam cubes was found to outperform the other MBSBRs. This was due to the fact that the attached-growth biomass within the 27-mL cubes was more shielded from the inhibitory effect of 4-CP than that of the smaller cubes and the mobility of 27-mL cubes was higher than that of the larger cubes. Thus, in treating high concentrations of 4-CP, the MBSBR packed with 27-mL PU foam cubes is the preferred system for the simultaneous removal of 4-CP and nitrogen.

CHAPTER 1

INTRODUCTION

The extensive discharge of nitrogen wastes from municipal, industrial and agricultural wastewaters without appropriate treatment can adversely contaminate the quality of receiving waters. Serious concern related to the adverse impacts of nitrogen wastes particularly ammonium-nitrogen (NH_4^+-N) includes dissolved oxygen (DO) depletion, toxicity, eutrophication and methemoglobinemia (Gerardi, 2002). Therefore, the necessity to remove NH_4^+-N in wastewaters prior to disposal is the utmost crucial to comply with the increasingly stringent discharged limits for nitrogen containing wastewaters.

Biological nitrogen removal has been broadly recognized to offer a cost saving and more environmentally friendly approach in removing NH_4^+-N via nitrification and denitrification processes (Gerardi, 2002; Jokela et al., 2002; Leta, 2004; Dempsey et al., 2005; Aslan et al., 2009). Over the years, various operational parameters had been studied with the intention to optimize the biological nitrogen removal (Deguchi and Kawashiwaya, 1994; Pochana and Keller, 1999; Katsogiannis et al., 2003; Daniel et al., 2009; Chu and Wang, 2011). However, the challenges in removing NH_4^+-N arise when toxic organic chemicals are present together in the NH_4^+-N containing wastewaters by which the nitrification process will be inhibited leading to the retardation of biological nitrogen removal (Kargi et al., 2005; Goh et al., 2009; Lim et al., 2012).

1.1 Biological Nitrogen Removal

The main path of biological nitrogen removal in treating NH_4^+-N containing wastewaters is via nitrification and denitrification processes. Nevertheless, in the presence of easily biodegraded organic substrates, the assimilation process may be responsible for the loss of nitrogen during which the NH_4^+-N is assimilated into the cell mass of the microorganisms. Previous attempts had shown that biomass assimilation could account for the removal of a large fraction of nitrogen when the concentration of organic matter in the influent was high (Lee et al., 2008; Rene et al., 2008). On the other hand, the ammonification process will result in the release of a portion of NH_4^+-N into the mixed liquor of the bioreactor due to the death and lysis of the cells (Grady et al., 1999).

1.1.1 Nitrification Process

The nitrification process is a two steps aerobic process. The first step involves the oxidation of NH_4^+-N by *Nitrosomonas* spp. to nitrite-nitrogen (NO_2^--N) which is subsequently oxidized to nitrate-nitrogen (NO_3^--N) in the second step mediated by *Nitrobacter* spp. (Kotlar et al., 1996; Qiao et al., 2008). The oxidation of NH_4^+-N to NO_2^--N and NO_2^--N to NO_3^--N are also conventionally known as nitritation and nitratation processes, respectively (Zeng et al., 2009; Fukumoto et al., 2011).

Nitritation process carried out by *Nitrosomonas* spp.:

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O \qquad (1.1)$$

Nitratation process carried out by *Nitrobacter* spp.:

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \qquad (1.2)$$

Overall nitrification process:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+ \qquad (1.3)$$

The nitrifying bacteria of *Nitrosomonas* spp. and *Nitrobacter* spp. are chemolithoautotrophs, signifying that the nitrifying bacteria obtain carbon from the inorganic carbon, i.e., carbon dioxide, and energy from the chemical reactions (Eqs. 1.1 and 1.2, respectively) for growth and multiplication. Carbon dioxide is made accessible to nitrifying bacteria in the form of bicarbonate ions in the wastewaters (Gerardi, 2002; Liao et al., 2008). As more energy can be harvested from the oxidation of NH_4^+-N to NO_2^--N than NO_2^--N to NO_3^--N, the population size of *Nitrosomonas* spp. is always larger than *Nitrobacter* spp. Besides, *Nitrosomonas* spp. also has a shorter generation time which enables its numbers to increase quickly in comparison with *Nitrobacter* spp. (Gerardi, 2002). Therefore, in the activated sludge system, the ability of being oxidized for NH_4^+-N is always higher than for NO_2^--N, causing a transient accumulation of NO_2^--N which disappears when NH_4^+-N is completely oxidized. However, during unfavourable operational conditions such as low DO concentration, excessive sludge washout, extreme change in temperature, etc., significant accumulation of NO_2^--N concentration can be observed in the mixed liquor of the bioreactors (Ruiz et al., 2006; Jubany et al., 2009; Lim et al., 2010; Gu et al., 2012).

Although the activated sludge system is used to treat NH_4^+-N containing wastewaters in many treatment plants, it is actually not an ideal system for the nitrification process. As shown by Gerardi (2002), the typical population size of nitrifying bacteria in the activated sludge system is only in the range of 3% to 10% with the remaining percentages consist of organotrophs. The nitrifying bacteria has a very low reproductive rate as relatively large amounts of NH_4^+-N and NO_2^--N are required during the assimilation process. Studies have proven that the nitrification process is affected by many condition variables including pH (Quinlan, 1984; Koren et al., 2000), temperature (Myers, 1975; Willers et al., 1993), DO (Stenstrom and Poduska, 1980; Ciudad et al., 2005), concentrations of ammonia and nitrous acid (Koren et al., 2000; Sudarno et al., 2011), chemical oxygen demand (COD)/N ratio (Carrera, et al., 2004; Fu, et al., 2009), salinity (Magalhaes et al., 2005; Aslan and Simsek, 2012), inorganic carbon (Wett and Rauch, 2003; Guisasola et al., 2007) as well as sludge retention time (SRT) (Lee et al., 2008; Wu et al., 2011). Thus, due to the poor growth of nitrifying bacteria, numerous researchers had suggested the optimum operational conditions for nitrification process to ensure its sustainability in the activated sludge system. Koren et al. (2000) had delineated that the optimum pH for nitrification process was 7.5-8.6. They also warned that a sufficient alkalinity must be supplied to neutralize the acid produced by the nitrification process to avoid a continuous reduction of pH which could adversely inhibit the nitrifying bacteria. Furthermore, Koren et al. (2000) had also reported the DO concentrations of more than 1.0 mg/L are needed for the occurrence of nitrification process. However, Guisasola et al. (2005) had pointed up the nitritation and nitratation oxygen affinity constants obtained in their study were 0.74 ± 0.02 and 1.75 ± 0.01 mg/L, respectively, which indicated that the nitratation process was more likely to be inhibited by low DO concentrations than nitritation process. As a result, the accumulation of NO_2^--N concentration could be observed due to the poor growth of nitrite oxidizing bacteria (NOB) in comparison with ammonium oxidizing bacteria (AOB) during the continuous operation with DO limitation condition (Zeng et al., 2011). In terms of the temperature, maximum nitrification rate was recorded at the range of 35-40 °C in the research performed by Willers et al. (1998). Sudarno et al. (2011) had later confirmed that both the maximum oxidation rate of NH_4^+-N and NO_2^--N were obtained at almost similar temperature range of 32.5-40 °C. In their study as well, a full recovery of the NH_4^+-N and NO_2^--N oxidation rates could be attained after the cold shock by increasing the temperature from 6 to 22.5 °C whereas, this recovery was not possible after the heat shock when the temperature was reduced from 50 to 22.5 °C. Concerning the SRT, longer SRT was necessary to provide an opportunity for the slow growing nitrifying bacteria to increase its population size and prevent it from being washed out of the bioreactors in order to improve the nitrification capability of the activated sludge system (Gerardi, 2002; Choi et al., 2008). Lee et al. (2008) demonstrated that the increase of SRT had led to the enhancement of nitrification process. Moreover, the effect of SRT on NH_4^+-N oxidation was also investigated by Aslan et al. (2009), which showed that the NH_4^+-N oxidation was completed in the shortest time with 40 days of SRT as compared with 30, 25 and 20 days. Patel et al. (2006) had also retrofitted the SRT of 45-50 days in their bioreactor while treating nitrogen containing wastewater to promote the nitrification process.

1.1.2 Denitrification Process

The sole nitrification process is only partially fulfilling the objective of biological nitrogen removal as the NH_4^+-N is merely converted to the oxidized nitrogen (NO_3^--N and NO_2^--N) by the nitrifying bacteria. The produced oxidized nitrogen must be subsequently reduced to the nitrogen gas (N_2) and released to the atmosphere in order to eliminate nitrogen from wastewaters. This reduction process is known as denitrification process which reduces the oxidized nitrogen to N_2 in the following sequence (Gerardi, 2002):

Nitrate ion (NO_3^-) → Nitrite ion (NO_2^-) → Nitric oxide (NO) → Nitrous oxide (N_2O) → Ntrogen gas (N_2)

The process of denitrification can be accomplished by a wide range of facultative anaerobes which make up an approximately 80% of the bacteria in the activated sludge system. The largest numbers of facultative anaerobes that are capable of performing the denitrification process are from *Alcaligenes*, *Bacillus* and *Pseudomonas* genera (Gerardi, 2002). Besides, the facultative anaerobes that denitrify are also termed by several names including denitrifying bacteria, denitrifiers, heterotrophs and organotrophs.

In assuring the viability of denitrification process, the carbon source must be made accessible to the denitrifying bacteria under an anoxic environment with DO concentrations lower than 1.0 mg/L or 2% saturation in the mixed liquor of the bioreactors (Goh, 2007; Lim et al., 2012). As the denitrifying bacteria can harvest more energy through the aerobic respiration rather than anoxic respiration, the denitrification process has to be strictly commenced in the anoxic environment to ensure the carbon source added is primarily used to reduce the oxidized nitrogen instead of being aerobically degraded (Gerardi, 2002). According to Gerardi (2002), the reduction of NO_3^- ion in the presence of carbon source under the anoxic environment can be expressed in two simplified biochemical reactions as below:

$$NO_3^- + \text{Carbon Source} \rightarrow NO_2^- + CO_2 + H_2O \qquad (1.4)$$

$$NO_2^- + \text{Carbon Source} \rightarrow N_2 + CO_2 + OH^- \qquad (1.5)$$

The denitrification process is as well being affected by the types and quantities of carbon source added. Eiroa et al. (2005) had validated that formaldehyde was a preferable carbon source for denitrification process in comparison with phenol as the consumption of phenol was only detectable after formaldehyde was depleted in the mixed liquor when both of these carbon sources were simultaneously used for denitrification process. In their study also, the inhibition of denitrification process was observed when the quantity of phenol added was increased. In contrast, Isaacs and Henze (1995) reported that the rate of denitrification increased with increasing quantity of sodium acetate added. In addition, the optimum pH for the denitrification process lies in the range of 7-8 albeit the ability of denitrifying bacteria to tolerate the pH between 6 and 9 had been reported (Dincer and Kargi, 2000; Koren et al., 2000). Alkalinity is also generated during the denitrification process which compensates approximately 50% of the alkalinity lost during the nitrification process (Gerardi, 2002; He et al., 2009). Hence, lesser buffer quantity is required to prevent the continuous decrease of pH when the nitrification process is executed together with the presence of denitrification process in the same bioreactor (Kulkarni, 2012).

1.2 Overview of 4-Chlorophenol

4-Chlorophenol (4-CP) with the synonyms of *p*-chlorophenol and 4-hydroxychlorobenzene, is broadly used in many industries mainly as an intermediate for further chemicals synthesis (Euro Chlor, 2002; Monsalvo et al., 2009; Sahoo et al., 2010; Li et al., 2011). Owing to high toxicity effects exerted by the 4-CP on environment and human health (ATSDR, 1999), the discharge of 4-CP containing wastewaters from the industries must be thoroughly treated before being released to receiving waters. Some of the fundamental physical and chemical properties of the 4-CP before the treatment processes are presented in Table 1.1.

1.2.1 Treatment of 4-CP Containing Wastewaters

The treatment of 4-CP containing wastewaters before discharging to receiving waters is vital to minimize the adverse effects of 4-CP towards the living organisms particularly human beings. Great efforts have been made by researchers from all over the world in studying the physical, chemical and biological processes or a combination of these processes to treat 4-CP containing wastewaters.

1.2.1.1 Physical Treatment Process

Among the various physical processes, adsorption process is the most commonly applied in treating 4-CP containing wastewaters. An assortment of adsorbents had been prepared from pistachio shells, aerobic granular sludge, rattan sawdust, straw, rubber tyres, copolymerization of styrenic monomer and divinylbenzene (DVB), Amberlite XAD-4 resin as well as zeolite are used for 4-CP adsorption studies (Streat et al., 1995; Oh et al., 2003; Bilgili, 2006; Kuleyin, 2007; Hameed et al., 2008; Tseng et al., 2010; Monsalvo et al., 2011). Although the adsorption process is effective in removing 4-CP from the wastewaters, the 4-CP is merely being adsorbed and concentrated on the adsorbent surfaces.

Table 1.1: Physical and chemical properties of 4-CP.

Parameter	Description	Reference
Molecular weight	128.56 g/mol	Mohamad (2011)
Chemical formula	C_6H_5ClO	ATSDR (1999)
Physical state	Crystal	Mohamad (2011)
Colour	White to pink	ATSDR (1999)
Odour	Medicinal odour	ATSDR (1999)
Melting point	43 °C	Euro Chlor (2002)
Boiling point	217 °C	Euro Chlor (2002)
Relative density	1.306 at 20 °C	Euro Chlor (2002)
Vapour pressure	0.51 hPa at 25 °C	Euro Chlor (2002)
Water solubility	27.1 g/L at 20 °C	Euro Chlor (2002)
pK_a	9.38 at 25 °C	Euro Chlor (2002)

1.2.1.2 Chemical Treatment Process

Chemical oxidation via ozonation process has a high oxidation potential to destroy toxic organic chemicals (Pi et al., 2007). The degradation of 4-CP by ozonation process had been investigated by many researchers around the world (Kuo, 1999; Sauleda and Brillas, 2001; Alvarez et al., 2005; Pi et al., 2007). Among the monochlorophenols, Kuo and Huang (1995) reported that 4-CP had the highest reactivity with the ozone with three moles of ozone required to completely rupture the aromatic ring of each mole of 4-CP. Nevertheless, the chlorine-containing intermediates formed from the aromatic ring cleavage were less reactive towards the presence of ozone, leading to the accumulation of these intermediates as the by-products of 4-CP ozonation process (Andreozzi and Marotta, 1999). A similar drawback was also observed in the electrochemical degradation in which the mineralization of 4-CP could only be attained at a certain degree. Wang and Wang (2007) demonstrated that incomplete removal of COD was detected although 100% removal efficiency of 4-CP was achieved during the electrochemical degradation of 4-CP using diaphragm electrolysis device.

1.2.1.3 Physical-Chemical Treatment Process

As single physical or chemical treatment process is incompetent in treating 4-CP containing wastewaters, many researchers choose to incorporate both physical and chemical methods to ensure a complete degradation of 4-CP; rather than physically transfer 4-CP from one phase to another or chemically oxidize 4-CP to only by-products level. Kuo and Wu (2010) had shown that the mineralization of 4-CP increased from 25% to 90% when solar assisted photo-Fenton process was applied in the treatment of 4-CP instead of unaided Fenton process. The presence of solar light in photo-Fenton process also promoted the reaction rate by a factor of 6.5 and 15.8 for COD and TOC degradation, respectively, as compared to Fenton process in their study. Furthermore, Liao and Kuo (2007) revealed that 100% degradation of 4-CP could be obtained when smectite-Ti catalyst was used in the photocatalysis process. The 4-CP degradation rate was also seen increasing with the increase of pH in their study as more OH^- ions were available at higher pH.

It is undeniable that a combination of physical and chemical treatment processes can improve the removal efficiency of 4-CP in the wastewaters in comparison with individual processes. However, the negative aspects of physical-chemical treatment process should not be taken too lightly. The cost of physical-chemical treatment process is generally high especially treating large quantity of 4-CP containing wastewaters. Moreover, the physical-chemical treatment process also involves tedious procedures (Gupta et al., 2000) and more treatment steps are required, e.g., during the preparation of catalyst in the catalysis process (Liao and Kuo, 2007; Lai et al., 2008). Besides, usually only low concentration ranges of 4-CP can be treated with physical-chemical methods in order to achieve complete mineralization (Catalkaya et al., 2003).

1.2.1.4 Biological Treatment Process

Despite the recalcitrant nature of 4-CP, great efforts are still being made towards the use of biological treatment process due to economical reasons and the ability of attaining complete mineralization of 4-CP in the wastewaters (Sahinkaya and Dilek, 2005; Monsalvo et al., 2009). Biological treatment process or simply bioremediation can basically be classified as either an aerobic or anaerobic process (Goh, 2007). According to Sahinkaya and Dilek (2005), aerobes are more efficient in degrading toxic organic chemicals because they grow faster than anaerobes and usually achieve complete mineralization of toxic organic chemicals, rather than transformation which gives rise to the accumulation of by-products as in the case of anaerobic treatment. Before the bioremediation process can be implemented in treating 4-CP containing wastewaters, the culture needs to be adequately acclimated in order to enhance its degradation capability of 4-CP (Moreno and Buitron, 2004). Sahinkaya and Dilek (2005) reported that although 4-CP removal was not observed with an unacclimated culture, almost complete removal was achieved with the acclimated culture up to 300 mg/L of 4-CP. It was also observed in their study that the toxicity of 4-CP on the culture decreased remarkably after the acclimation process. When the bioremediation process is carried out by the acclimated culture, the 4-CP in the wastewater is primarily biodegraded via the *meta*-cleavage pathway (Farrell and Quilty, 1999). The cleavage of the 4-CP's aromatic ring will result in the formation of 5-chloro-2-hydroxymuconic semialdehyde which is later completely mineralized by the acclimated culture (Farrell and Quilty, 1999; Sahinkaya and Dilek, 2006).

Various biological treatment systems have been designed and developed for the removal of 4-CP, e.g., external-loop fluidized bed airlift bioreactor, rotating brush biofim reactor, rotating tubes biofilm reactor and rotating biological contactor systems (Loh and Ranganath, 2005; Eker and Kargi, 2006; Sahinkaya and Dilek, 2006a; Eker and Kargi, 2010; Ma et al., 2012). Among the systems, sequencing batch reactor system is widely used in degrading 4-CP in the wastewaters (Buitron et al., 2005; Sahinkaya and Dilek, 2007; Monsalvo et al., 2009). However, the advantage of retrofitting attached-growth process in the sequencing batch reactor has received increasing attention among researchers recently.

1.3 Sequencing Batch Reactor (SBR) System

For decades, SBR system has been applied for the treatment of domestic, municipal and industrial wastewaters and also offers an attractive option in the biological wastewater treatment systems with the following advantages (Irvine and Ketchum, 1989; Wobus et al., 1995; Louzeiro et al., 2002; Goh, 2007):

- Simplicity and cost effective;
- Capability of combining aerobic and anoxic phases in a single reactor;
- Flexibility in terms of sequence and cycle time;
- Ideal quiescent settling condition;
- Highly resistant to influent loading fluctuation.

As an activated sludge system, SBR is basically running on the fill and draw principle. The operation of the SBR is usually accomplished by five sequential periods, namely FILL, REACT, SETTLE, DRAW and IDLE in a single tank rather than simultaneously in the separate tanks which are typically observed in the conventional activated sludge system (Arora et al., 1985). Thus, this permits the building of a small treatment plant and is suitable in the area with limited land available.

1.3.1 Removal of Nitrogen via SBR

As discussed in Sections 1.1.1 and 1.1.2, biological nitrogen removal is attained through the nitrification and denitrification processes which require aerobic and anoxic conditions, respectively. With the SBR system, both aerobic and anoxic conditions can be flexibly programmed during the REACT period to boost the biological nitrogen removal process. In most of the practices, the REACT period of the SBR system is initiated with the aerobic condition to promote the nitrification process. Once all of the NH_4^+-N is oxidized, the aeration system is terminated to nurture the anoxic condition which will stimulate the denitrification process (Goh, 2007; Lim et al., 2010). Nevertheless, the application of intermittent aeration (IA) strategy during the REACT period of the SBR system has been extensively studied for further enhancement of NH_4^+-N removal (Cheng and Liu, 2001; Li et al., 2008; Bernat et al., 2011; Li et al., 2011a). In this strategy, the aeration system is alternately being switched on and off to allow the aeration and non-aeration periods to be periodically repeated. As a result, the nitrification and denitrification process will be periodically taking place as well and the excessive accumulation of the oxidized nitrogen can be avoided (Zeng et al., 2008). Cheng and Liu (2001) verified that the pattern of 1 h of aeration period followed by 1 h of non-aeration period was the best IA strategy compared to the others for nitrogen removal. With this IA strategy, NH_4^+-N removal efficiency to the tune of 92% could be achieved in their research.

The challenge of the application of IA strategy for nitrogen removal arises when treating wastewaters containing low COD/N ratio, e.g., supernatant from sludge digesters, mature landfill leachate and pretreated industrial wastewater by anaerobic fermentation. The limited amount of carbon source content in these wastewaters will hamper the denitrification process during the non-aeration period which inhibits the reduction of the oxidized nitrogen to N_2. Li et al. (2011a) had investigated the nitrogen removal via the SBR operated with the IA strategy in treating wastewater containing low COD/N ratio. During the non-aeration period, the concentration of NO_2^--N was observed to remain stable in their study which was plausibly due to the exhausted supply of carbon source.

1.3.2 Simultaneous Removal of 4-CP and Nitrogen via SBR

Many researches had been conducted to assess the removal of 4-CP via the SBR system (Buitron et al., 2005; Sahinkaya and Dilek, 2007; Monsalvo et al., 2009; Carucci et al., 2010). Sahinkaya and Dilek (2006) had evaluated the effect of biogenic substrate (peptone) concentrations on 4-CP removal and found that decreasing the peptone concentrations did not affect the 4-CP degradation profiles in the SBR. High COD removal efficiencies (90-95%) and complete 4-CP removal were also observed in their study even in the absence of peptone. In the research to determine the influence of the origin of the inoculum on the degradation of 4-CP via SBR, Moreno and Buitron (2004) had claimed that the activated sludge coming from a municipal wastewater treatment plant presented an initial higher bacterial diversity than the ones coming from the domestic and industrial wastewater treatment plants. Consequently, the municipal wastewater treatment plant's activated sludge had a better adaptability to the presence of 4-CP in the wastewater.

High concentrations of phenolic compounds and NH_4^+-N are commonly found in the petrochemical and industrial wastewaters (Texier and Gomez, 2007; Silva et al., 2011). As 4-CP is highly toxic and recalcitrant in nature, it often poses problems in the biological nitrogen removal via SBR. The nitrification process is usually inhibited in the presence of 4-CP, leading to the NH_4^+-N remaining not oxidized and appearing in the treated effluent. Kargi et al. (2005) revealed that the removal efficiencies of COD, NH_4^+-N and 4-CP in the SBR decreased to only 76%, 72% and 34%, respectively, on the addition of 200 mg/L of 4-CP. They therefore recommended that the SBR should be operated at the 4-CP concentration of less than 100 mg/L to achieve relatively high nitrogen and 4-CP removal. Moreover, although up to 400 mg/L of 4-CP was completely mineralized in the investigation performed by Mohamad (2011) using SBR, the NH_4^+-N removal efficiency was seen decreasing from 100% in the absence of 4-CP to only 36% when the added 4-CP was 400 mg/L, indicating the inhibitory effects of 4-CP on the nitrifying bacteria.

1.4 Moving Bed Sequencing Batch Reactor (MBSBR) System

For years, various attempts have been committed to modify the SBR system in order to improve the performance. Among others, the MBSBR which is developed by introducing support media into the SBR has attracted much interest among researchers in the field of biological wastewater treatment (Garzon-Zuniga and Gonzalez-Martinez, 1996; Sirianuntapiboon and Yommee, 2006; Goh et al., 2009; Hosseini Koupaie et al., 2012). In general, three different types of support media, namely Kaldnes (polyethylene media), Liapor (ceramic media) and Linpor (plastic media with high porosity) are used to pack the MBSBR (Valdivia et al., 2007). The mobility of the added support media in the MBSBR is usually maintained by the aeration or mechanical mixing systems (Odegaard et al., 1994). Once the attached-growth biomass or biofilm has grown on the surfaces of support media, the MBSBR which incorporates both suspended-growth and attached-growth biomasses is expected to perform better than SBR which consists of only suspended-growth biomass.

1.4.1 Removal of Nitrogen via MBSBR

Among the three different types of support media used, Valdivia et al. (2007) reported that the MBSBR packed with Linpor performed better than either Kaldnes or Liapor in removing COD and NH_4^+-N at the organic loading rate of over 3.0 g COD/m^3 day. The polyurethane (PU) foam which belongs to the Linpor type of support media has a high porosity and is an ideal medium for biomass immobilization through attachment. Besides, PU foam also has a good mechanical strength and is relatively low cost (Golla et al., 1994; Chu and Wang, 2011).

Nitrogen removal through simultaneous nitrification and denitrification (SND) process had been investigated in previous studies (Kotlar et al., 1996; Daniel et al., 2009; Chu and Wang, 2011). The SND process is potentially reducing the operational period which ultimately results in the reduction of operation cost and time. The oxygen concentration and the availability of the carbon source for denitrification were found to be the important parameters for the commencement of SND process (Pochana and Keller, 1999). By using the PU foam cubes as a support media for biofilm formation, studies had demonstrated the occurrence of decreasing dissolved oxygen (DO) gradient within the inner layer of biofilm and also deep inside the PU foam which leads to the creation of an anoxic zone (Morper, 1994; Guo et al., 2010). Furthermore, as the carbon source is also an important constituent to ensure the feasibility of the denitrification process, the SND process is frequently inhibited during treatment of wastewaters containing low COD/N ratio. Nevertheless, in the study of Guo et al. (2010), the SND process was still detected in the anoxic zone of the PU foam. The occurrence of the SND process without the addition of the external carbon source in their study could be explained by the possibility of carbon storage in the deeper biofilm layers which was reported by several researchers (Morgenroth and Wilderer, 1999; Pastorelli et al., 1999; Gieseke et al., 2002). The presence of carbon storage together with anoxic zone within the biofilm and deep inside the PU foam could be used to kindle the denitrification process which eventually stimulated the SND process.

Based on the results of previous studies, PU foam was found to be a good support media in the MBSBR system. Its porous structure will allow the formation of attached-growth biomass, the establishment of anoxic zone and the storage of carbon which enhance the nitrogen removal via SND process. However, to date, relatively little is documented on the effect of different sizes of the PU foam cubes on the nitrogen removal in the MBSBR system. In addition, the biomass growth onto the surfaces and into the interior porous structure of the various sizes of PU foam cubes along with the role of these various sizes of PU foam cubes in the SND process in treating low COD/N ratio wastewaters have not been thoroughly investigated.

1.4.2 Integration of IA Strategy in MBSBR (IA-MBSBR)

The main advantage of IA application in treating nitrogen containing wastewaters includes the reduction of the operational cost due to a decrease of continuous aeration (CA) supply and the amount of external carbon source required for the subsequent denitrification phase (Li et al., 2008). In the IA system, the high DO concentration during the aeration period enables the aerobic nitrifiers to oxidize NH_4^+-N to NO_2^--N and then to NO_3^--N. During the subsequent non-aeration period, the DO concentration will decrease to such a low level that the NO_2^--N and NO_3^--N species are reduced to N_2 mediated by the anoxic denitrifiers (Li et al., 2008).

Nonetheless, as discussed in Section 1.3.1, the practicability of IA strategy application in removing nitrogen from low COD/N ratio wastewaters is still questionable due to inadequate supply of carbon source which results in the incompetency of denitrification process. In view of the carbon storage in the deeper layers of biofilm within the support media which can serve as the carbon source (Section 1.4.1), the integration of IA strategy in MBSBR (IA-MBSBR) packed with

PU foam cubes is not expected to face the aforesaid problem. The carbon storage is not only found in the biofilm on the external surfaces which is typically exhibited by most of the support media but is also located within the interior porous structure of the PU foam cubes which is being occupied by the attached-growth biomass. However, to date, the available information to sustain the viability of IA-MBSBR in the enhancement of nitrogen removal is still lacking. The availability of the carbon storage located onto and into the acclimated PU foam cubes with attached-growth biomass also needed to be further verified.

1.4.3 Alternative Solid Carbon Source for Denitrification Process Enhancement in IA-MBSBR

Liquid carbon sources such as ethanol, methanol, acetate or glucose are normally added during the denitrification process. However, the disadvantages of this approach are the risk of overdosing which would deteriorate the effluent quality and the requirement of the use of a sophisticated and costly process control (Hiraishi and Khan, 2003; Zhou et al., 2009; Shen and Wang, 2011). In recent years, the use of solid carbon sources as an alternative to liquid carbon sources has gained increasing interest among the researchers (Robinson-Lora and Brennan, 2009; Wang and Wang, 2009; Zhou et al., 2009; Zhou et al., 2009a; Shen and Wang, 2011; Fan et al., 2012). The solid carbon sources packed in the bioreactors perform two important tasks, namely to serve as a reducing agent in denitrification process and to act as a support media for biofilm formation (Wang and Wang, 2009; Zhou et al., 2009a). The presence of constant carbon sources and anoxic zones within the deeper layers of the biofilm as well as in the porous structure of the solid carbon sources would ensure a stable reduction of the oxidized nitrogen (Walters et al., 2009; Wang and Wang, 2009; Zhou et al., 2009a). Hence, the use of an expensive and sophisticated system control can be avoided as the addition of liquid carbon sources is no longer necessary. Generally, two types of solid carbon sources, namely synthetic and natural polymers have been studied. Synthetic polymers include polycaprolactone (PCL) (Boley et al., 2000; Honda and Osawa, 2002; Zhou et al., 2009), polylactic acid (PLA) (Fan et al., 2012), polyhydroxyalkanoates (PHA) (Boley et al., 2000; Hiraishi and Khan, 2003) and bionolle (Boley et al., 2000) whereas, natural polymers include wheat straw (Soares and Abeliovich, 1998; Fan et al., 2012), cotton (Rocca et al., 2005), biodegradable meal box (Wang and Wang, 2009) and crab-shell chitin (Robinson-Lora and Brennan, 2009). Since the rate of denitrification is closely related to the biodegradability of the solid carbon source (Hiraishi and Khan, 2003), the use of natural polymers which are more likely to be biodegraded than synthetic polymers is expected to attain higher denitrification rates than synthetic polymers. In fact, Wang and Wang (2009) had proved that the rate of denitrification by using biodegradable meal box was higher than PCL. Fan et al. (2012) had also revealed that faster biofilm development and higher denitrification rate could be achieved when wheat straw was utilized as a solid carbon source as compared to PLA. From an economic point of view, the relatively high cost of using synthetic polymers such as PCL and bionolle limits its extensive application especially in treating large quantity of wastewater (Walters et al., 2009; Chu and Wang, 2011a). Zhou et al. (2009) estimated that a denitrification system using polyhydroxybutyrate (PHB) costs almost ten times higher than a traditional system using methanol. As natural polymers such as cotton and wheat straw are easily available and they can achieved higher denitrification rate than synthetic polymers, the application of solid carbon source using natural polymers have gained priority over that of synthetic polymers.

Nevertheless, in order to maintain the efficiency of denitrification, the addition of fresh natural polymers after certain period of operation is necessary. Soares and Abeliovich (1998) reported that a weekly addition of fresh wheat straw could be seen preventing the deterioration of denitrification performance. Moreover, for some natural polymers such as crab-shell chitin, the content of soluble components such as volatile fatty acids and chloride, sulphate and ammonium ions are very high. Robinson-Lora and Brennan (2009) illustrated that an initial flushing period of 3 days was needed before the crab-shell chitin could be used as a solid carbon source to reduce the concentrations of soluble components in the treated effluent. Consequently, as a result of frequent addition or replacement of such solid carbon sources, more time is required to be spent on flushing. In addition, post-treatment system such as trickling sand filter or powdered activated carbon adsorption have to be installed to remove colour and organic matter released particularly from fresh natural polymers (Soares and Abeliovich, 1998; Rocca et al., 2005). Thus, it is essential to find an alternative natural material which can serve as a solid carbon source without releasing soluble components.

Qiao et al. (2008) reported that the potential of attached-growth biomass in oxidizing NH_4^+-N was lower than the suspended-growth biomass as the AOB's mobility was more restricted in the attached state, leading to lessened contact possibility between AOB and NH_4^+-N. For this reason, the attached-growth biomass formed onto and into the PU foam cubes in the IA-MBSBR could be utilized to serve as a solid carbon source for the denitrification process. These days, the research activities have only focused on the reuse of suspended-growth biomass or sludge as an internal carbon source (Ra et al., 2000; Kampas et al., 2007; Soares et al., 2010). The use of dried attached-growth biomass as a solid carbon source has not been reported in the literature. Therefore, the possibility of using dried attached-growth biomass formed onto and into the PU foam cubes as a solid carbon source for the denitrification process in the IA-MBSBR system deserves more research attentions.

1.4.4 Simultaneous Removal of 4-CP and Nitrogen via MBSBR

Among various biologically sound treatment processes for 4-CP containing wastewaters, MBSBR has generated much interest among researchers recently (Moreno-Andrade et al., 2009; Buitron and Moreno-Andrade, 2011; Mohamad, 2011). The MBSBR system which exploits the advantages of suspended-growth and attached-growth processes is certainly more sustainable in removing high concentrations of 4-CP from wastewaters as compared with the typical SBR system. The biofilm system in the MBSBR is generally more resistant to high concentrations of toxic compounds because of higher biomass concentrations and the existence of diffusion barriers within the biofilm for the toxic compounds (Eker and Kargi, 2006). Moreno-Andrade et al. (2009) reported that the specific degradation rate of 4-CP decreased by only 8% when a shock load of 4-CP at the concentration of 1050 mg L^{-1} was introduced in to the MBSBR. However, with similar 4-CP shock load concentration, the specific degradation rate of 4-CP via SBR was observed to decrease by 70% (Buitron et al., 2003).

As pointed out in Section 1.3.2, the use of SBR treatment system has suffered from low removal efficiency of NH_4^+-N in the presence of 4-CP together with NH_4^+-N in the wastewaters. In the study carried out by Mohamad (2011), the NH_4^+-N removal efficiencies in the MBSBR packed with only 3% of PU foams cubes were always higher than the SBR for all the 4-CP concentrations experimented which once again illustrated the superiority of MBSBR in the simultaneous removal of 4-CP and nitrogen over the SBR. Nevertheless, in IA-MBSBR, although the IA is the preferred strategy for nitrogen removal as discussed in Section 1.4.2, the application of this aeration strategy would impede the performance of aerobes in degrading 4-CP particularly during the non-aeration period (Armenante et al., 1999). As a result, the nitrification process would be inhibited as well due to the presence of non-degraded 4-CP. For this reason, the aeration strategy ranging from intermittent to continuous aeration needs a comprehensive assessment in order to avoid the inhibition of simultaneous removal of 4-CP and nitrogen in the MBSBR due to the application of inappropriate aeration strategy.

Since the 4-CP is categorized as a toxic organic chemical, increasing concentration will generally increase the toxicity level which will inhibit the nitrification and hinder its own degradation processes (Kargi et al., 2005; Mohamad, 2011). As the biofilm system is more resistant to toxic compounds, the treatment of high concentrations of 4-CP is usually executed using biological system augmented with support media of relatively high surface areas for biofilm formation in order to reduce the toxicity and improve the degradation of 4-CP. Wang et al. (2005) provided the evidence that increasing the support media concentrations from 10% to 50% which meant increasing the media's surface areas for biofilm formation, had led to the increase of NH_4^+-N and COD removal rates. Another approach which can increase the surface areas of support media for biofilm formation without altering the packing volume is reducing the sizes of support media. As the information regarding the treatment of 4-CP and nitrogen using MBSBR packed with various sizes of PU foam cubes as support media is rather scanty in the literature, it is essential to investigate the effect of sizes of PU foam cubes on simultaneous removal of 4-CP and nitrogen in MBSBR. In the presence of increasing 4-CP concentrations, the capability of MBSBR packed with various sizes of PU foam cubes in the simultaneous removal of 4-CP and nitrogen is also deemed crucial to be evaluated to affirm the viability of using smaller size of PU foam cubes which possess larger surface areas for biofilm formation.

1.5 Kinetic Models of NH_4^+-N and 4-CP Removal

The empirical studies of NH_4^+-N and 4-CP removal are assumed to follow the zeroth-order kinetics and the rate is expressed by the following equation:

$$\frac{d[C]}{dt} = -k \tag{1.6}$$

Integration of this differential equation gives the following equation:

$$[C] = -kt + [C]_o \tag{1.7}$$

where $[C]$ is the concentration of either NH_4^+-N or 4-CP (in mg/L) at particular time, $[C]_O$ is the initial concentration of either NH_4^+-N or 4-CP (in mg/L), k is the pseudo-zeroth-order rate constant of either NH_4^+-N or 4-CP removal (in mg/L min) and t is the time course (in min).

Since 4-CP biodegradation by microorganism has generally been known to be inhibited by 4-CP itself, Haldane model is frequently selected to represent the kinetics removal of inhibitory substrates due to its mathematical simplicity and wide acceptance. The 4-CP removal kinetics was described according to the Haldane model as follows: