



UNIVERSITAT AUTÒNOMA DE BARCELONA

**DEPARTAMENT D'ENGINYERIA QUÍMICA
ESCOLA TÈCNICA SUPERIOR D'ENGINYERIA**

**TREATMENT OF COMPLEX INDUSTRIAL
WASTEWATERS CONTAINING AMMONIUM
AND PHENOLIC COMPOUNDS USING
GRANULAR SLUDGE IN CONTINUOUS AIRLIFT
REACTORS**

PhD Thesis

ZULKIFLY BIN JEMAAT

June 2013

Summary

The simultaneous nitrification and phenolic compounds removal using aerobic granular reactors in continuous mode was studied in this thesis. The study is divided into two main subjects. The first one is devoted to the modeling of nitrification, while the other part is dedicated to the experimental study of simultaneous nitrification and phenolic compounds removal.

In the modeling study, a mathematical biofilm model was developed to describe nitrification in aerobic granular reactors operating in continuous mode. The model incorporated a [DO]/[TAN] ratio control strategy to maintain the proportion between the concentrations of dissolved oxygen (DO) and total ammonia nitrogen (TAN) in the reactor effluent to a desired value. The model was validated with a large set of experimental results previously reported in the literature, as well as, data gathered from laboratory and pilot plant granular reactors treating reject water. The model was used to study the effect of: a) DO and TAN setpoints, b) operating temperature, c) biofilm characteristics (granules size, density) and d) ammonium concentrations in the influent on the achievement of full nitrification. The results indicated that full nitrification was stably maintained and enhanced by applying the [DO]/[TAN] ratio control strategy in the operation of aerobic granular sludge reactors. Moreover, the model predicted that aerobic granules size larger than 1.5 mm and high ammonium concentrations in the influent enhanced the achievement of stable full nitrification. Furthermore, at low temperature, full nitrification with granular reactors was demonstrated to be possible. On the contrary, poor influence of the biofilm density on the achievement of full nitrification was found with the simulation study.

In the experimental study, an airlift reactor was employed. The airlift reactor was inoculated with granular sludge performing biological nutrient removal. A synthetic wastewater containing a high-strength ammonium concentration ($950 \pm 25 \text{ mg N L}^{-1}$) was fed into the airlift reactor that was operated until partial nitrification was obtained. Once partial nitrification was achieved, the airlift reactor was bioaugmented with *p*-nitrophenol (PNP)-degrading activated sludge to enhance the growth of phenols-degraders in the nitrifying granules. Immediately, *o*-cresol (up to 100 mg L^{-1}) or PNP (up to 15 mg L^{-1}) were progressively added to the high-strength ammonium influent with the objective of studying the simultaneous partial nitrification and phenols removal.

In the study of simultaneous partial nitrification and *o*-cresol removal, stable partial nitrification process was maintained for more than 100 days of operation. Moreover, full biodegradation of *o*-cresol was achieved during the whole experimental period. Also, *o*-cresol shock load events were applied and the partial nitrification process was kept stable and unaffected during these events. The achieved nitrogen loading rate (NLR_v) and *o*-cresol loading rate (oCLR_v) were ca. $1.1 \text{ g N L}^{-1}\text{d}^{-1}$ and $0.11 \text{ g o-cresol L}^{-1}\text{d}^{-1}$, respectively. Analysis of fluorescent in-situ hybridization (FISH) indicated that *Acinetobacter* genus, betaproteobacterial ammonia-oxidizing bacteria (β AOB) and *Nitrobacter* sp. were identified into the granules. The operation of the reactor was continued to perform an experiment devoted to assessing its performance under three sequentially alternating pollutant (SAP) scenarios. In each one of the SAP scenarios, 15 mg L^{-1} of a secondary phenolic compound (i.e. *p*-nitrophenol (PNP), phenol or 2-chlorophenol (2CP)) was added to the regular influent composed of ammonium and *o*-cresol (the primary phenolic compound) for a short period of time (between 20 to 25 days). The results illustrated that partial nitrification and *o*-cresol biodegradation were maintained without exhibiting any sign of inhibition by the presence of PNP or phenol.

However, when 2CP was present in the influent, 90 % of the partial nitrification and 25 % of the *o*-cresol degradation were inhibited within two days. In spite of this massive failure, the reactor could be rapidly re-activated, fully recovering the partial nitrification and *o*-cresol removal (the primary recalcitrant compound) capacity in 14 and 4 days, respectively. These findings demonstrate that treatment of complex industrial wastewaters with highly variable influent composition could be feasible in a continuous aerobic granular sludge reactor.

In the study of simultaneous nitrification and PNP removal, nitrification was maintained for producing an effluent suitable for heterotrophic denitrification. However, during the first 175 days, PNP biodegradation was unstable and several accumulation episodes occurred. Oxygen limiting condition was found to be the main explanation for these events. The increase in dissolved oxygen (DO) concentration in the reactor from 2 to 4.5 mg O₂ L⁻¹ permitted to achieve complete and stable PNP removal till the end of the experimental period. The achieved NLR_V and PNP loading rate (PNP-LR_V) were ca. 1.0 g N L⁻¹d⁻¹ and 16 mg PNP L⁻¹d⁻¹, respectively. The performance of the reactor was further assessed by performing two starvation studies: i) PNP starvation and ii) total starvation period (temporary stop). Results show that full recovery of PNP degradation was achieved within 2 days after the PNP starvation period ended, while full recovery of simultaneous nitrification and PNP removal was accomplished in just 14 days after the restart of the reactor.

In conclusion, the use of continuous aerobic granular reactors for the simultaneous nitrification and phenolic compounds removal is feasible. Aerobic granules were proven to be resistant and resilient to the shock loads, to the alternating presence of recalcitrant compounds and to the starvation periods that are situations frequently found in industrial wastewater treatment plants due to changes in the industrial production schedules. In the near future, we propose the simultaneous nitrification and phenolic compounds removal should be combined either with heterotrophic denitrification or Anammox for sustainable nitrogen removal.

Resumen

Esta tesis doctoral se centra en el estudio de la nitrificación y la eliminación de compuestos fenólicos de manera simultánea mediante el uso de biomasa granular aerobia en reactores continuos tipo *airlift*. El estudio está dividido en dos partes principales. La primera, trata sobre la modelización de la nitrificación, mientras que la segunda está dedicada al trabajo experimental sobre nitrificación y eliminación de compuestos fenólicos.

En el estudio de modelización, se desarrolló un modelo matemático de biopelícula para describir la nitrificación en reactores de biomasa granular aerobia operando en continuo. El modelo incorpora una estrategia de control de relación entre la concentración de oxígeno disuelto (DO) y la de nitrógeno total en forma amoniacal (TAN) en el efluente del reactor ($[DO]/[TAN]$). El modelo se validó con un gran número de datos experimentales previamente publicados en la bibliografía, así como también con datos obtenidos del tratamiento del agua de rechazo en reactores granulares a escala laboratorio y piloto. El modelo se utilizó para estudiar el efecto sobre la obtención de la nitrificación de: a) los puntos de consigna de DO y de TAN, b) la temperatura de operación, c) las características de la biopelícula (tamaño de partícula, densidad) y d) la concentración de amonio en el afluente. Los resultados obtenidos indicaron que la aplicación de la estrategia de control de relación $[DO]/[TAN]$ para la operación del reactor de biomasa granular aerobia contribuyeron a mantener una nitrificación completa de forma estable, potenciando su obtención de forma rápida y sencilla. Además, el modelo contribuyó a determinar que los gránulos aerobios mayores a 1.5 mm y las altas concentraciones de amonio en el afluente potenciaban la obtención de la nitrificación completa de forma estable. La densidad de biopelícula, sin embargo, se estableció como un factor con poca influencia sobre el proceso. Además, se demostró que era posible obtener la nitrificación total de manera estable a bajas temperaturas con reactores de biomasa granular.

En el trabajo experimental, se utilizó un reactor continuo tipo *airlift* y biomasa granular aerobia. Para la puesta en marcha del reactor, se utilizó como inóculo la biomasa granular procedente de un reactor que realizaba eliminación de nutrientes. Como alimento del reactor se utilizó un agua residual sintética con un alto contenido de amonio ($950 \pm 25 \text{ mg N L}^{-1}$). El reactor se operó hasta la obtención de la nitrificación parcial. Una vez obtenida la nitrificación parcial, el reactor se bioaumentó con un lodo activo que contenía biomasa degradadora de *p*-nitrofenol (PNP) para potenciar el crecimiento de microorganismos degradadores de fenoles en los gránulos nitrificantes. Acto seguido y mientras el reactor trataba una carga elevada de amonio, se añadieron progresivamente al afluente *o*-cresol (hasta 100 mg L^{-1}) o PNP (hasta 15 mg L^{-1}), con el objetivo de estudiar la nitrificación parcial y la eliminación de *o*-cresol o de PNP de manera simultánea.

En el estudio de la nitrificación parcial simultánea a la eliminación de *o*-cresol, se mantuvo estable el proceso de nitrificación parcial durante más de 100 días de operación. Además, se obtuvo una biodegradación completa de *o*-cresol durante todo el periodo experimental. También se realizaron choques de carga de *o*-cresol, durante los cuales el proceso de nitrificación parcial se mantuvo estable y sin ninguna afectación evidente causada por dichos eventos. Las cargas volumétricas obtenidas de nitrógeno (NLR_V) y de *o*-cresol ($oCLR_V$) fueron de $1.1 \text{ g N L}^{-1} \text{ d}^{-1}$ y de $0.11 \text{ g } o\text{-cresol L}^{-1} \text{ d}^{-1}$,

respectivamente. El análisis mediante hibridación fluorescente *in situ* (FISH) permitió la identificar la presencia de betaproteobacterias amonio-oxidantes (β AOB), bacterias del género *Acinetobacter* y *Nitrobacter sp.* Posteriormente, se continuó con la operación del reactor, y se llevó a cabo un experimento relacionado con el funcionamiento del reactor bajo tres escenarios de alternancia secuencial de contaminantes (SAP). En cada uno de estos escenarios SAP se añadieron al afluente 15 mg L^{-1} de un compuesto fenólico secundario (es decir PNP, fenol o 2-clorofenol (2CP)) por un corto periodo de tiempo (entre 20 y 25 días). Los resultados ilustraron que se mantuvo la nitrificación parcial y la biodegradación de *o*-cresol sin que se detectara ningún signo de inhibición de la actividad biológica por la presencia de PNP o de fenol. Sin embargo, durante el escenario SAP con 2CP en el afluente se registró una inhibición del 90% de la nitrificación parcial y del 25% de la degradación de *o*-cresol en solo dos días. A pesar de este fallo masivo del reactor, este pudo ser rápidamente reactivado, recuperando completamente la nitrificación parcial y la eliminación de *o*-cresol (el compuesto primario recalcitrante) en 14 y en 4 días, respectivamente. Estos resultados demuestran que es factible el tratamiento de aguas residuales industriales complejas con una alta variabilidad en la composición del afluente mediante el uso de reactores continuos de biomasa granular.

En el estudio de la nitrificación simultánea a la eliminación de PNP, se mantuvo la nitrificación durante la mayor parte del periodo operacional, obteniéndose un efluente adecuado para la desnitrificación heterotrófica. Sin embargo, durante los primeros 175 días, la biodegradación de PNP fue inestable, observándose diversos episodios de acumulación de PNP. Esta acumulación se determinó que era debida a las condiciones limitantes de DO. El incremento en la concentración de DO en el reactor de 2 a $4.5 \text{ mg O}_2 \text{ L}^{-1}$ permitió obtener una eliminación completa y estable del PNP hasta el final del periodo experimental. La NLR_v y la carga de PNP (PNP-LR_v) que se obtuvieron fueron de $1.0 \text{ g N L}^{-1} \text{ d}^{-1}$ y $16 \text{ mg PNP L}^{-1} \text{ d}^{-1}$, respectivamente. Además, se evaluó el funcionamiento del reactor realizando dos estudios de hambruna: i) hambruna de PNP y ii) hambruna total (parada del reactor). Los resultados mostraron una recuperación total de la degradación del PNP en sólo 2 días después de la finalización del primer periodo de hambruna, mientras que se consiguió una recuperación total de la nitrificación y de la eliminación del PNP en sólo 14 días después de volver a poner en marcha el reactor (finalización del segundo periodo de hambruna).

Se puede concluir, por lo tanto, que es factible el uso de reactores de biomasa granular aerobia para la nitrificación y la eliminación de compuestos fenólicos de forma simultánea. Así mismo, se ha demostrado que la biomasa granular aerobia es resistente a los choques de carga puntuales, a la presencia alternante de compuestos recalcitrantes y a los periodos de hambruna, siendo muy frecuente encontrar estas condiciones en plantas de tratamiento de aguas residuales industriales, debido a los cambios en la planificación de la producción del proceso que genera el agua residual. En un futuro próximo, se propone que la nitrificación simultánea a la eliminación de compuestos fenólicos podría combinarse tanto con la desnitrificación heterotrófica como con el proceso Anammox para una eliminación sostenible del nitrógeno.

Thesis outline

The thesis is divided into four main parts. The main content of each part and the corresponding chapters will be detailed as follows.

Part I (General Introduction) consists of three chapters i.e. Chapters 1, 2 and 3. In Chapter 1, an overview of the state of the art in the field of biological nitrogen and phenolic compounds removal from industrial wastewaters is presented. Special emphasis was paid to ammonium oxidation by the 'nitrite route' i.e. nitrification and to aerobic biodegradation of phenolic compounds. The technology of aerobic granular sludge was also highlighted for the treatment of nitrogen and phenolic compounds. In Chapter 2, the main goal and objectives of the thesis are pointed out and elaborated. Finally, in Chapter 3, a general overview of the materials and methods used in the experimental study are described. However, the specific information regarding materials and methods corresponding to each one of the experiments performed are described in detail in each one of the corresponding chapters.

In Part II (Modeling of Nitrification in Granular Reactors), Chapter 4 and 5 are presented. In Chapter 4, a mathematical biofilm model was developed to describe nitrification using an aerobic granular sludge reactor operating in continuous mode. The implementation of a control strategy based on the ratio of Dissolved Oxygen (DO) concentration to Total Ammonia Nitrogen (TAN) concentration was tested in a real application of a continuous reactor of aerobic granular sludge was incorporated into the model and simulated. Several scenarios were assessed to investigate the stability and robustness of the applied control strategy. The model was validated with experimental results from pilot plant nitrifying granular reactors treating a high-strength-ammonium concentration wastewater. Furthermore, the model was used to study the achievement of full nitrification in front of changes in DO and TAN setpoints, in operating temperature and in granule size. Additionally, the importance of controlling the TAN concentration was highlighted with different scenarios, in which periodic disturbances were applied mimicking poor control situations. In Chapter 5, the previous developed model was further explored for study the effect of ammonium concentrations in the influent and granular sludge density on the achievement of full nitrification using aerobic granular sludge reactors. The model was validated with experimental results from lab-scale and pilot plant nitrifying granular reactors treating high-strength-ammonium concentration wastewaters. The results of the modeling study are discussed in detail, in Chapter 5. Prior to the experimental study in Part III, the developed model was utilised for doing a pre-feasibility study of the simultaneous nitrification and phenolic compounds removal. Several scenarios were designed and simulated to determine the granular reactor start-up for simultaneous nitrification and phenolic compounds removal.

In Part III (Simultaneous Nitrification and Phenolic Compounds Removal), Chapter 6, 7, 8 and 9 are presented. Chapter 6 presents the feasibility study of simultaneous partial nitrification and *o*-cresol removal using a continuous airlift reactor of aerobic granular sludge. In addition, the performance and stability of the reactor in front of two shock loading events were also assessed and discussed. In Chapter 7, the aerobic granular reactor performing simultaneous partial nitrification and *o*-cresol removal was tested against several scenarios of Sequentially Alternating Pollutant (SAP). In each of the SAP scenarios, a secondary phenolic compound was introduced in the influent for a period of 3 weeks. The secondary phenolic compounds selected were phenol, *p*-nitrophenol (PNP) and 2-chlorophenol. In Chapter 8, the feasibility study of simultaneous nitrification and PNP removal using the aerobic granular sludge reactor operating in continuous mode was performed. Further in Chapter 9, the aerobic granular

reactor performing simultaneous nitrification and PNP removal was submitted to starvation periods. Two starvation scenarios were performed: i) PNP starvation and ii) PNP and TAN starvation, i.e. reactor was completely stopped. The subsequent reactivation of the biological processes was evaluated and discussed in this chapter.

Finally, Part VI (Conclusions) gives an overview of the main achievements of this thesis and points out the topics for future research derived from this study. Figure 1 illustrates the general outline of the thesis through a graphical abstract.

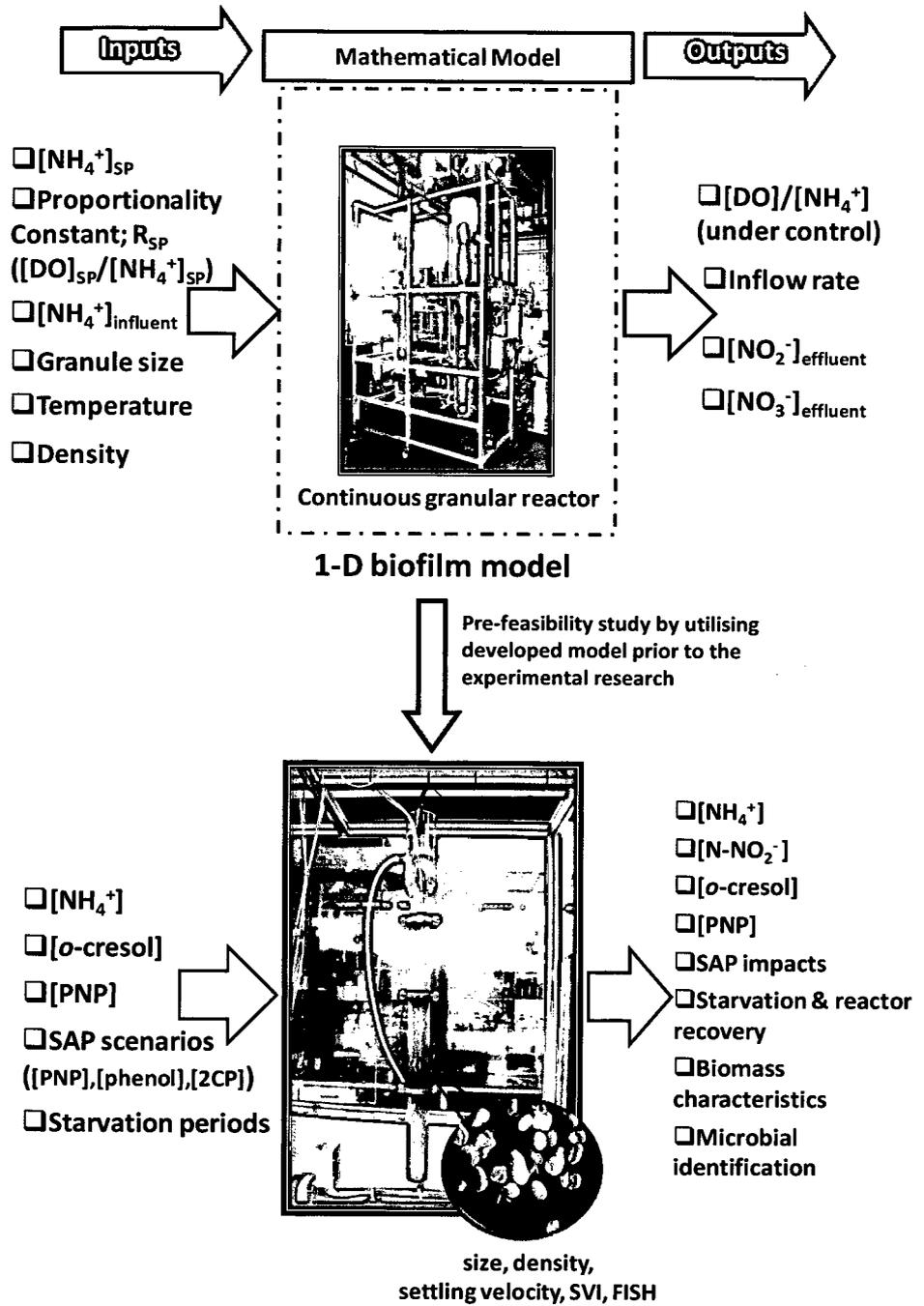


Figure 1 General overview of the thesis from the mathematical modeling of nitrification to the subsequent experimental research.

Table of Contents

PART I GENERAL INTRODUCTION

Chapter 1 Introduction	1
1.1 Industrial wastewater effluent and its challenge	2
1.2 Biological wastewater treatment	6
1.2.1 Biological nitrogen removal	7
1.2.2 Advanced biological nitrogen removal	10
1.2.3 Mathematical modeling in biological nitrogen removal	14
1.2.4 Biological phenolic compounds removal	17
1.3 Aerobic granular sludge	26
1.4 Future perspectives on simultaneous removal using aerobic granular sludge	29
1.5 References	36
Chapter 2 Objectives	54
2.1 Problem statement	55
2.2 Objectives	55
Chapter 3 Materials and methods	57
3.1 Experimental set-up	58
3.2 Wastewater characteristics	59
3.3 Analytical methods	60
3.4 Biomass characterization	61
3.5 Microorganisms ecology assessment	62
3.6 References	64

PART II MODELING OF NITRITATION IN GRANULAR REACTORS

Chapter 4 Closed-loop control of ammonium concentration in nitrification: Convenient for reactor operation but also for modeling	66
4.1 Introduction	67
4.2 Materials and methods	69
4.2.1 Reactor set-up and wastewater	69
4.2.2 Analytical methods	71
4.3 Model description	71
4.3.1 Biofilm model, kinetics and parameters	71
4.3.2 Modeling the TAN control loop inside the ratio control strategy	72
4.3.3 Simulation strategy for model validation	73
4.3.4 Scenarios to test the importance of the control of ammonium concentration	74
4.4 Results and discussion	74

4.4.1 Model validation	74
4.4.2 Highlighting the effects of the closed-loop control	75
4.4.3 Effect of the DO/TAN concentration ratio on the applied loading rate	77
4.4.4 Effect of granule size on $R_{max,fN}$	80
4.4.5 Effect of temperature on $R_{max,fN}$ at different granule sizes	82
4.5 Conclusions	83
4.6 References	83

Chapter 5 The achievement of high rate nitrification with aerobic granular sludge reactors enhanced by sludge circulation events

5.1 Introduction	87
5.2 Materials and methods	88
5.2.1 Experimental set-ups, wastewater and inoculums	88
5.2.2 Analytical methodology	88
5.2.3 Model development	89
5.2.4 Ratio control strategy	89
5.3 Results and discussion	90
5.3.1 Reactor operation	90
5.3.2 Influence of the influent TAN concentration	92
5.3.3 Influence of biofilm density on the achievement of full nitrification	94
5.4 Conclusions	95
5.5 References	95

PART III SIMULTANEOUS NITRIFICATION AND PHENOLIC COMPOUNDS REMOVAL

Chapter 6 Partial nitrification and *o*-cresol removal in a continuous airlift reactor using granular sludge

6.1 Introduction	97
6.2 Materials and methods	98
6.2.1 Experimental set-up and reactor conditions	99
6.2.2 Wastewater composition	99
6.2.3 Inoculum and reactor operation before bioaugmentation	100
6.2.4 Bioaugmentation, and operational strategy	100
6.2.5 Analytical methods	101
6.2.6 FISH analysis	101
6.3 Results and discussion	102
6.3.1 Performance of the granular airlift reactor for simultaneous removal of ammonium and <i>o</i> -cresol	102
6.3.2 <i>o</i> -Cresol shock load events	106
6.3.3 Identification of dominant species by FISH	108
6.4 Conclusions	109

6.5 References	109
Chapter 7 Performance of simultaneous partial nitrification and <i>o</i>-cresol biodegradation in a continuous aerobic granular sludge reactor in front of sequentially alternating pollutant (SAP) scenarios	113
7.1 Introduction	114
7.2 Materials and methods	115
7.2.1 Reactor set-up and operating conditions	115
7.2.2 Wastewater	115
7.2.3 Description of the sequentially alternating pollutant (SAP) scenarios	116
7.2.4 Analytical methods	117
7.3 Results and discussion	117
7.3.1 Effect of PNP as secondary phenolic compound	117
7.3.2 Effect of phenol as secondary phenolic compound	121
7.3.3 Effect of 2CP as secondary phenolic compound	123
7.4 Conclusions	126
7.5 References	127
Chapter 8 Simultaneous nitrification and <i>p</i>-nitrophenol (PNP) removal using granular sludge in a continuous airlift reactor	130
8.1 Introduction	131
8.2 Materials and methods	132
8.2.1 Experimental set-up and reactor conditions	132
8.2.2 Wastewater composition	133
8.2.3 Inoculum and reactor operation before bioaugmentation	133
8.2.4 Bioaugmentation and the operational strategy	133
8.2.5 Analytical methods	134
8.2.6 Determination of the oxygen half saturation coefficient, $K_{O_{PNP}}$ for PNP-degraders and the AOB inhibition coefficient for PNP, $K_{I_{PNP,AOB}}$	135
8.2.7 FISH analysis	136
8.3 Results and discussion	137
8.3.1 Performance of the granular airlift reactor for simultaneous removal of ammonium and PNP	137
8.3.2 Morphological changes and identification of dominant species using FISH technique	142
8.4 Conclusions	145
8.5 References	145

Chapter 9 Starvation and subsequent reactivation of an aerobic granular reactor treating simultaneously ammonium and <i>p</i>-nitrophenol	149
9.1 Introduction	150
9.2 Materials and methods	151
9.2.1 Reactor set-up, operations and wastewater	151
9.2.3 Starvation and reactivation experiments	152
9.2.4 Analytical methods	152
9.3 Results and discussion	153
9.3.1 Reactor performance before, during and after the PNP starvation period and subsequent reactivation of PNP degradation	153
9.3.2 Reactor performance after the total starvation period	157
9.4 Conclusions	158
9.5 References	159
 PART IV CONCLUSIONS	
 Chapter 10 General conclusions and future perspectives	162
10.1 Conclusions	163
10.2 Future research	164
 Appendix	166

PART I
GENERAL INTRODUCTION

Introduction

Summary

In this chapter, an overview of the state of the art with regard to industrial wastewaters and challenges in their treatment is presented focussing the discussion on certain industries producing wastewaters containing ammonium and phenolic compounds. A detailed literature review related to the biological treatment of nitrogen and phenolic compounds using either activated or aerobic granular sludge was performed and presented in table format. Finally, a brief discussion on the future perspectives on the simultaneous removal of ammonium and phenolic compounds using aerobic granular sludge technology is highlighted.

1.1 Industrial wastewater effluent and its challenge

It is known that industrial sectors are vital for the world economy. Industries are spurring and becoming the backbone of the most developed countries' economy for instance Japan, Germany and United States. However, do you ever realise that the industrial sector, besides producing useful products, it is certainly generating a significant portion of the wastewater discharged worldwide? Direct discharge of industrial wastewaters into rivers, lakes and coastal areas has resulted in serious environmental pollution problems, causing negative effects in various ecosystems on which human life relies on. There are many types of industrial wastewaters; each industrial sector generates its own particular combination of pollutants. The amount of wastewater generated depends on the technical level of the process in each industrial sector and could be gradually reduced with the improvement of industrial technologies. The increasing rates of industrial wastewater in developing countries are thought to be much higher than those in developed countries, in fact, it has been predicted that, in the early 21st century, the industrial wastewater pollution problem will shift from the developed countries to the developing countries (Shi, 2009). Therefore, the industrial pollution problems faced by different countries worldwide are different.

Typically, industrial wastewaters may contain high levels of inorganic, organic pollutants, which can be easily biodegradable, but whose impact load on the ecosystems, either in chemical oxygen demand (COD) or total suspended solids (TSS) may be in the tens of thousands mg L⁻¹ (Ng, 2006). Industrial wastewaters also may contain recalcitrant and toxic compounds, which are difficult to treat biologically. Table 1.1 presents typical industrial pollutants found in the wastewaters of certain industrial sectors. The inorganic and COD compounds are the main sources of pollutants in most of the industrial sector listed (Table 1.1). In particular, sectors from petrochemical, coke and chemical industry, their wastewaters may be characterised by high concentration of organic, ammonia and phenolic compounds (Table 1.2). Typically, the presence of phenolic compounds in wastewaters is characterised by the total phenols concentration. According to published data of the European Pollutant Release and Transfer Register (E-PRTR), the total amount of phenols released by the EU reporting states to E-PRTR was 1032 tonnes/year (phenols as total C) in 2010 (Table 1.3) (European Environment Agency (EEA), 2010). From this total amount of phenols released, about 74 % was contributed from petroleum, refinery, coke and chemical sectors. In addition, about 8 % of total nitrogen released by the EU reporting states was also contributed from these sectors (Table 1.3). Common phenolic compounds found in such industrial wastewaters are phenol, nitrophenols, cresols and chlorinated phenols (Silva et al., 2011). Phenolic compounds are known to be toxic, carcinogenic and mutagenic to aquatic organisms at low concentrations (EPA, 1994). If these compounds enter to wastewater treatment facility, especially in the case of the biological treatment unit and an appropriate monitoring is not in place, they could inhibit the existing biological processes. Moreover, ammonia compound in industrial wastewaters may cause serious environmental problems if this compound is not properly removed before discharge into the receiving water bodies. A too high nitrogen concentration in the receiving waters can lead to eutrophication, hypoxia and loss of biodiversity and habitat (Galloway et al., 2003, 2008).

At present, wastewaters from petrochemical, coke and chemical industry are treated by a combination of physicochemical and biological processes. Figure 1.1 illustrates the general schemes of wastewater treatment plants in these industries. The typical wastewater treatment plants includes: i) physical processes for the removal of oil, grease and heavy hydrocarbons ii) physicochemical processes for the removal of macromolecular, colloidal substances, non

biodegradable organic (recalcitrant and toxic compounds) and iii) biological processes aiming to remove biodegradable organics, nutrients (ammonia and phosphorus) and particularly short-chain hydrocarbons (Tobiszewski et al., 2012). In the biological processes, systems like activated sludge, membrane bioreactor, aerated lagoon, trickling filters, nitrification or/and denitrification are most widely used. In certain cases, the effluent after biological processes is further treated in a tertiary treatment (sand filtration, chemical oxidation) for post-treatment prior to discharge or even for reusing purposes. Conventional physicochemical treatment processes have several drawbacks associated, for instance high energy demand, high operating costs, problems with by-products disposal and often; these methods cannot sufficiently remove several of the toxic and recalcitrant contaminants. In the case of physicochemical process that is partially removed recalcitrant/toxic compounds, when a biological treatment is subsequently installed, the biological activity could be inhibited by the presence of these compounds and consequently, may complicate the efficiency of the wastewater treatment plant.

Table 1.1 Typical industrial wastewater pollutant characteristics, sorted by industrial sectors (Shi, 2009)

Sectors	Pollutants
Iron and steel	COD, oil, metals, acids, phenols, cyanides
Textiles and leather	COD, solids, sulphides, chromium, ammonia, dyes
Pulp and paper	COD, solids, chlorinated organic compounds, phenols
Petrochemicals and refineries	COD, mineral oils, phenols, ammonia, chromium, aromatics
Chemicals	COD, organic chemicals, heavy metals, suspended solids, cyanides, phenols, aromatics, ammonia
Non-ferrous metals	Fluorine, suspended solids
High-technology (e.g: microelectronics, optoelectronics, semiconductor)	COD, ammonia, organic chemicals, suspended solids
Mining	Suspended solids, metals, acids, salts
Coke and gas	COD, organic chemicals, ammonia, phenols, cyanides
Pharmaceutical	COD, organic chemicals, aromatics, ammonia

Table 1.2 Typical compounds present in wastewaters from petrochemicals and refineries, chemicals and coke and gas sector. Chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solids (TSS).

Sectors	Compound concentration (g L ⁻¹)					Reference
	COD	BOD	Ammonia	Phenols	TSS	
Petrochemicals and refineries	0.07 -1.0	0.01-0.36	0.02 – 0.07	0.01-0.2	0.02-0.6	(Diya'uddeen et al., 2011)
Chemicals	9.6 – 17	1.4 - 4.3	0.6 – 1.7	0 – 5	6 – 10.3	(Abeliovich, 1985; UNEP, 1998)
Coke and gas	0.9-3.1	0.5-1.4	0.5-2.2	0.01 -0.5	0.02-3.3	(Chang et al., 2008)

Table 1.3 The amount of total nitrogen and phenols releases to water in the year 2010 according to industrial activity as reported by the European Pollutant Release and Transfer Register E-PRTR (EEA, 2010).

No	Industrial activity	Total nitrogen (tonnes)	Phenols (as total C) (tonnes)
1	Energy sector (e.g. petrochemical, coke, oil & gas)	6,803	728
2	Production and processing of metals (e.g. ferrous, non-ferrous, metal ore)	8,557	39.3
3	Mineral industry	1,165	0.765
4	Chemical industry (e.g. organic, inorganic, pharmaceutical)	25,968	34.4
5	Waste and waste water management (including urban waste water treatment plant)	346,900	212.7
6	Paper and wood production processing	6,418	15.2
7	Intensive livestock production and aquaculture	38,717	0.259
8	Animal and vegetable products from the food and beverage sector	3,855	0.447
9	Other activities (e.g. dyeing of fibres, textiles)	238	0.311
	Total	438,620	1,031

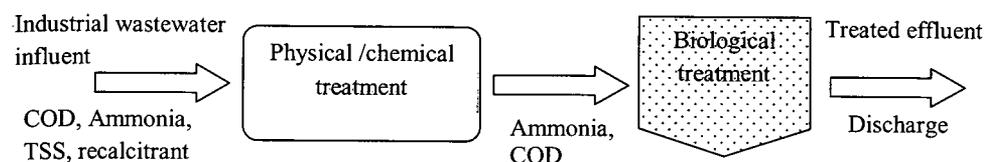


Figure 1.1 A general wastewater treatment scheme in petrochemicals and refineries industry.

On top of that, several difficulties or operational changes encountered by industries during the production and processing activities could cause the biological treatment methods of industrial wastewater treatment plants to be uncertain and ineffective (Sipma et al., 2010). Such difficulties faced by the biological treatment due to the instability of industrial activities are:

- i) Operational problems, caused by pH, temperature, salinity changes
- ii) High organic loads, shock loads and toxic loads
- iii) Presence of sequentially alternating pollutants (SAP) due to a variable production schedule
- iv) Long-term starvation periods due to maintenance or minor upgrading purposes
- v) Presence of toxic compounds due to the change of process route or acquiring new technology to meet customers' demand and requirements

Often, fluctuation in wastewater characteristics can be dampened by the provision of a flow equalization or buffer tank prior to the biological process (Sipma et al., 2010). In many cases, WWTPs are normally run at a more constant flow rate because of such storage reservoirs provided in the system (Droste, 1997). Although flow equalization is determined based on hydraulics rather than concentrations, it often prevents biological downstream processes from shock loads or high concentrations of toxic chemicals and may further provide in-system for pH neutralization, minimizing the consumption of chemicals (Tchobanoglous et al., 2004). However, highly variable concentrations of different components will make it impossible to achieve a uniform loading for all components (Droste, 1997). Because of such variability, flow equalization is likely of limited effectiveness. Moreover, weekly or monthly alternated production schemes that generate distinct wastewater characteristics, and for which flow equalization is ineffective, ensures the need for a robust treatment processes capable to cope with considerable fluctuations (Sipma et al., 2010).

Besides, the industries also need to ensure that the treated effluent is meeting the standards for water discharge set by the government or local authorities before it can be released to the receiving body. Nowadays, more stringent legislation governing the discharge of effluents has been imposed by many countries, aiming to ensure that our environment is well protected. For instance, ammonia and phenol were set below 50 mg N L^{-1} and 1 mg L^{-1} , respectively under RD 849/1986 (Spanish Government, 1986). RD 849/1986 is imposed to any effluent discharges from urban wastewater treatment plant and a specific industry (as listed in the RD 849/1986). As an example of a South East Asian Country, the Malaysian Government has enforced a standard limit below 20 mg N L^{-1} for ammonia and 1 mg L^{-1} for phenols for industrial effluent quality under the Environmental Quality Regulations 2009 (DOE, 2009). It indicates that the stricter regulations and laws have been enforced worldwide regarding the standards for wastewater discharge limits that must be fulfilled by industries

before the effluent can be discharged into receiving bodies. It also illustrates the efforts and concerns of most of the countries in the world regarding the environmental issues for sustaining a healthy environment.

Therefore, the need to adopt advanced treatment processes, including aerobic granular sludge are crucial either to ensure the quality standards for discharged water abide by legislation as well as to overcome the difficulties faced by industries on the current treatment methods employed.

The application of aerobic granular sludge to treat various types of industrial wastewaters including petrochemical and chemical industry is proven feasible and practical (Adav et al., 2009; Maszenan et al., 2011). Compared to conventional activated sludge systems, the granular biomass could retain a higher biomass concentration in the reactor and avoid biomass washout occurrence due to its excellent settleability, compact and dense microbial structure (Gao et al., 2011a). Moreover, a diversified microbial aggregate within the granule structure provides a platform for simultaneous removal of several contaminants (Adav et al., 2008). Granular biomass are also resilient to toxic compounds, shock loads events and its biological processes could be reactivated and recovered easily after a long-term starvation periods (Gao et al., 2011a). Above all, the application of aerobic granular sludge to treat complex industrial wastewaters has been demonstrated to be useful and promising in the industrial wastewater treatment. Nevertheless, further research is needed to explore the possibility of using the same granular biomass for degrading different recalcitrant compounds, including its ability to withstand in front of instabilities of the upstream process (SAP events and long-term starvation periods). The efforts will fill the gaps relating to the application of aerobic granular technology; so that it will proliferate completely, providing the opportunity to deal with water pollution and sustain a healthy environment.

1.2 Biological wastewater treatment

The overall objective of the biological treatment of industrial wastewater is to remove or reduce the concentration of organic and inorganic compounds. The organic compounds may include proteins, carbohydrates, oils, fats and synthetic organic molecules such alkenes, aromatics, alcohols and volatile organic compounds. Meanwhile, the inorganic compounds include ammonia, nitrite, nitrate, sulphur and phosphorus (Tchobanoglous et al., 2004). Aerobic biological treatment is employed for removal of biodegradable COD and the stabilization of organic matter found in industrial wastewaters using processes such as activated sludge, trickling filter or the rotating biological contactor (Tchobanoglous et al., 2004). Anaerobic processes are sometimes employed before aerobic processes for the treatment of high-strength, readily biodegradable wastewaters. However, other organics (non biodegradable, recalcitrant compounds) are difficult to treat biologically and often; physicochemical methods (i.e. advanced oxidation processing, distillation, adsorption), as explained before, are employed. Biological nitrogen removal, both nitrification and denitrification, is employed for removal of ammonia from wastewaters. While this process is predictable in the case of municipal wastewaters, many industrial wastewaters are inhibitory to the nitrifying organisms.

In an aerobic biological process, for instance, microorganisms are used to oxidise the dissolved and particulate carbonaceous organic matter into simple end products (i.e. CO₂, H₂O) and additional biomass (Figure 1.2). Microorganisms are also used to remove nitrogen and phosphorus in wastewater treatment processes. Specific bacteria are capable of oxidizing

ammonium to nitrite and nitrate (nitrification), while other bacteria can reduce the oxidized nitrogen to gaseous nitrogen. For phosphorus removal, biological processes are configured to encourage the growth of bacteria with the ability to take up and store large amounts of inorganic phosphorus and subsequent solids separation.

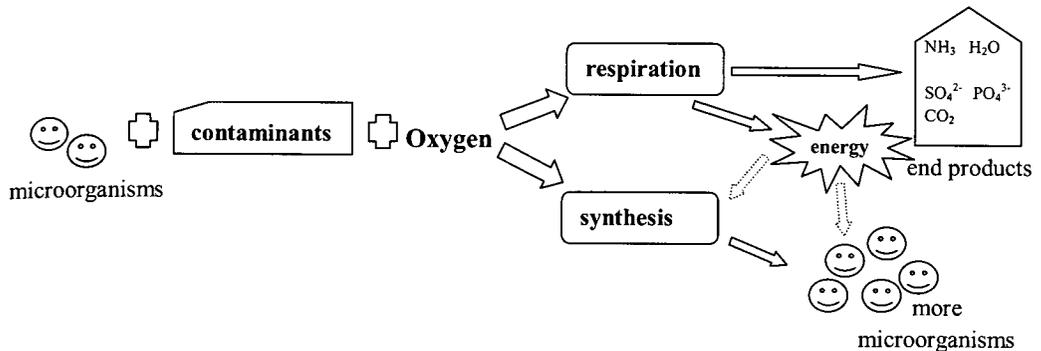


Figure 1.2 Aerobic oxidation of contaminants dissolved in wastewater by microorganisms and their products produced. Adapted from Tchobanoglous et al. (2004).

1.2.1 Biological nitrogen removal

The untreated wastewater can contain nitrogen in the form of organic nitrogen, ammonia (NH₃-N), nitrite (NO₂-N) and nitrate (NO₃-N). These nitrogen compounds can be removed by physicochemical or biological processes. The complete biological nitrogen removal can be accomplished in an aerobic (ammonium oxidation) and anaerobic condition (either with Anammox or heterotrophic denitrification). Biological nitrogen removal involves initial conversion of nitrogen contained in the wastewater to NO₃-N and then converts the NO₃-N to inert nitrogen gas (N₂). Low-cost biological nitrogen removal technologies have proved to be very effective in removing ammonia from industrial wastewater (Bagchi et al., 2012). Such novel processes including partial nitrification, aerobic denitrification, SHARON (Single reactor High activity Ammonia Removal over Nitrite), anaerobic ammonium oxidation (Anammox), and its combined system (for example, completely autotrophic nitrogen removal over nitrite, CANON). However, most ammonium-rich wastewaters are produced with a certain concentration range of organics (e.g., old landfill leachates), sometimes both recalcitrant and toxic compounds, sulfides, phosphates, and other inhibitory compounds that could upset the autotrophic bacteria which are mostly responsible in all low-cost biological nitrogen-removing technologies (Bagchi et al., (2012).

The biological nitrogen cycle is complex and plays an important role in the environment. This cycle is recently re-classified according to potential biochemical pathway as shown in Figure 1.3. Besides, the ammonium concentration in wastewater, the COD/N ratio also plays an important role for the selection of suitable biological nitrogen removal (Vázquez-Padín, 2009). For instance, in the case of COD/N > 20, the assimilation of nitrogen by heterotrophic bacteria is sufficient to remove nitrogen. In the case of 20 < COD/N < 5, the removal of nitrogen can be carried out by assimilation and nitrification-denitrification pathway. Finally, for wastewater containing COD/N < 5 ratio, the nitrogen removal by nitrite route processes is preferred (partial nitrification-denitrification, or partial nitrification-Anammox). In this sense, a combined system for nitrogen removal based on partial nitrification with Anammox has various

advantages, such as no need for external carbon addition, negligible sludge production and less energy and oxygen requirements than the conventional process (Jetten et al., 2002).

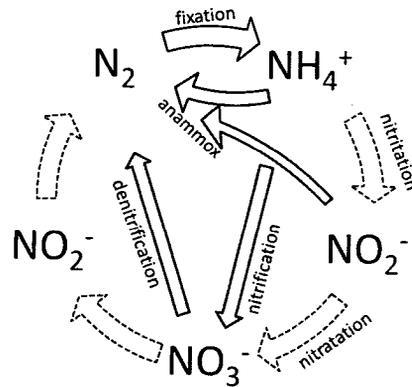
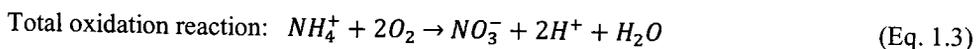
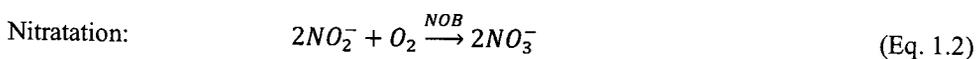
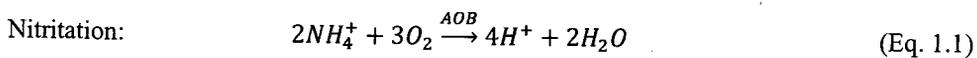


Figure 1.3 Biological transformations in the nitrogen cycle. Adapted from van Benthum (1998).

Nitrification

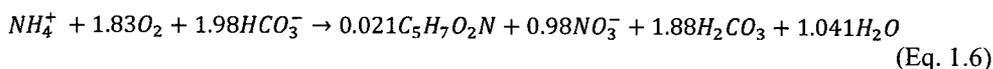
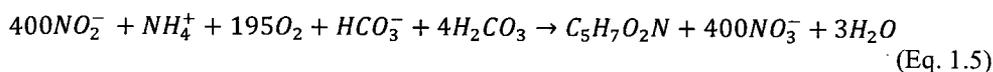
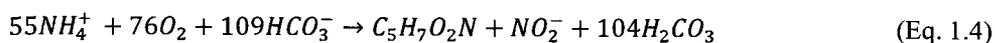
Nitrification is the biological oxidation of ammonia into nitrite followed by oxidation of nitrite into nitrate through the action of two phylogenetically independent groups of autotrophic aerobic bacteria, namely, ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB). The nitrification process is carried out in two sequential stages: the ammonia oxidation to nitrite (nitritation) and the subsequent oxidation of nitrite to nitrate (nitratation). It should be noted that the two groups of autotrophic bacteria are distinctly different. Starting with classical experiments on nitrification by Winogradsky (1891), the bacteria genera commonly noted for nitrification in wastewater treatment are the autotrophic bacteria *Nitrosomonas* and *Nitrobacter*, which oxidize ammonia to nitrite and then to nitrate, respectively. Other autotrophic bacteria genera capable of obtaining energy from the oxidation of ammonia to nitrite are *Nitrosococcus*, *Nitrospira*, *Nitrosolobus* and *Nitrosovibrio* (Painter, 1970). Besides *Nitrobacter*, nitrite can also be oxidized by other autotrophic bacteria as *Nitrococcus*, *Nitrospira*, *Nitrospina* and *Nitroeystis* (Wagner et al., 1995).

The energy-yielding two-step oxidation of ammonia to nitrate is as followed:



Based on the above total oxidation reaction, the oxygen required for complete oxidation of ammonium is 4.57 g O_2 /g N oxidized with 3.43 g O_2 /g N used for nitrite production and 1.14 g O_2 /g N oxidized. When synthesis is considered, the amount of oxygen required is less than

4.57 g O₂/g N. The following reaction steps are applied when the biomass growth and the bicarbonate used as carbon source are being considered in the oxidation.



Based on global reaction (Eq. 1.6), it will be noted that nitrification:

- i) Requires 4.18 g O₂ for each g of ammonium (as N) oxidized
- ii) 0.15 – 0.17 g of new cells are formed, where the bacteria composition is expressed as C₅H₇O₂N.
- iii) In term of alkalinity, 8.92 g of bicarbonate is needed for each g of ammonium (as N). The quantity of inorganic carbon could be a limiting factor during nitrification.
- iv) pH of the system tends to decrease due to alkalinity removal.

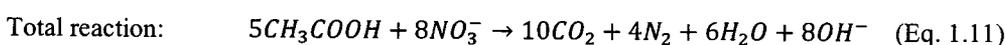
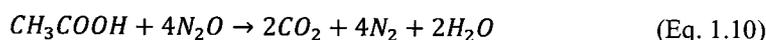
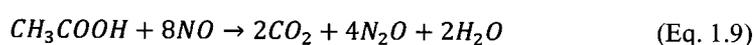
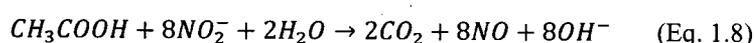
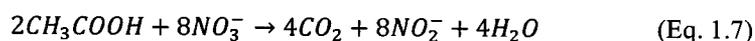
Maintaining nitrification would be relatively easy for WWTPs when operating in steady state. However, the desired steady state conditions often do not exist in the WWTP environment. For instance, all treatment plants experience significant fluctuation in daily flow, organic loading, nitrogen loading, temperature and concentrations of toxic chemicals. The nitrification process can be easily upset and lose efficiency with the numerous changes that could occur on a daily basis. In the worst case, the treatment facilities could violate its ammonium discharge limit. These upsets may last only a short period (a few days or weeks) or can be chronic problems. In general, several factors need to be monitored to ensure the successful of the nitrification process: temperature, pH, dissolved oxygen, sufficient ammonia concentration, organic load, presence of toxic compounds, etc.

Denitrification

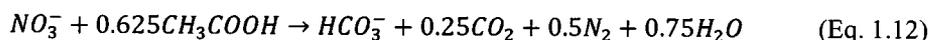
Denitrification process is the reduction of nitrate and nitrite to nitrogen gas in the absence of oxygen (anoxic conditions). Nitrite and nitrate act as electron acceptors. Biological denitrification is an integral part of the BNR, which involves both nitrification and denitrification. Bacteria capable of denitrification is both, heterotrophic and autotrophic. The heterotrophic organisms include the following genera: *Achromobacter*, *Acinetobacter*, *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Chromobacterium*, *Moraxella*, *Pseudomonas*, *Rhizobium* among others (Payne, 1981). In addition, Gayle et al., (1989) listed *Halobacterium* and *Methanomonas*. *Pseudomonas* are the most common and widely distributed of all denitrifiers, and have been shown to use a wide array of organic compounds as carbon source including methanol, carbohydrates, organic acids, alcohols, benzoates, and other aromatic compounds (Payne, 1981). Most of these bacteria are facultative aerobic organisms with the ability of using oxygen, as well as, nitrate or nitrite, and some of them can also carry out fermentation in the absence of nitrate or oxygen. These groups of organisms also can grow heterotrophically if an organic carbon source is present (Gayle et al., 1989). At present, the heterotrophic denitrification is the most common and widely used in WWTPs

Autotrophic nitrifying bacteria, such as *Nitrosomonas europaea*, can use nitrite to oxidize ammonia, with the production of nitrogen gas, when dissolved oxygen is not present (Bock et al., 1995). With oxygen present, these bacteria oxidize the ammonia with oxygen as the electron acceptor. Ammonia oxidation with the reduction of nitrite under anaerobic conditions has been shown at temperatures above 20 °C in the Anammox process, which was discovered in the mid-1990s (Strous et al., 1997). The bacteria in Anammox process are different from the autotrophic nitrifying bacteria described above, in that it cannot use oxygen for ammonia oxidation (Jetten et al., 1999). The Anammox bacteria could not be isolated and grown in pure culture (Strous et al., 1999), but an enrichment was obtained by density purification for 16S rRNA extraction and analysis. Phylogenetic analysis showed that the bacteria is in the order *Planctomycetales*, a division with the domain Bacteria. Under anaerobic conditions, the ammonia oxidation rate by the Anammox bacteria was shown to be 6 to 10 times faster than that for *N. Europaea* (Jetten et al., 1999). Recently, Anammox process for nitrogen removal from urban or industrial wastewaters has gained a tremendous research interest in the scientific community.

As explained before, the heterotrophic denitrification process requires the presence of a source of organic carbon as an electron donor and then, nitrate would act as the last electron acceptor in the respiratory chain substituting the oxygen. The reduction is carried out by subsequent steps through different oxidation states of nitrogen and the global stoichiometry with acetic acid as organic carbon source (for example) is represented in the following reaction steps;



Rewriting Eq. 1.11 taking into account the equilibrium of CO_2 gives Eq. 1.12.



From the Eq. 1.12, it can be inferred that the denitrification process originates an increase in the medium alkalinity and that 40% of the organic matter needed is used to reduce nitrate to nitrite.

1.2.2 Advanced biological nitrogen removal

It is known that nitrification involves two sub-processes; oxidation of ammonia to nitrite and oxidation of nitrite to nitrate. In general, the presence of nitrite is undesired in wastewater treatment or other domains; however, some recent advanced BNR processes prefer nitrite as an intermediate (Sinha and Annachhatre, 2006). The so-called 'nitrite route' is a shortened nitrification process until nitrite, and then subsequent denitrification of the nitrite to nitrogen gas. The need of treatment for high-strength ammonium-concentration wastewater has motivated the research of new processes and optimal configurations. These novel treatments

i.e. partial nitrification, SHARON (Single reactor High activity Ammonia Removal over Nitrite), Anammox, CANON (Completely Autotrophic Nitrogen removal Over Nitrite), OLAND (Oxygen-Limited Autotrophic Nitrification-Denitrification), aerobic deamonification is briefly described in this section. The general principle of these processes is illustrated in Figure 1.4.

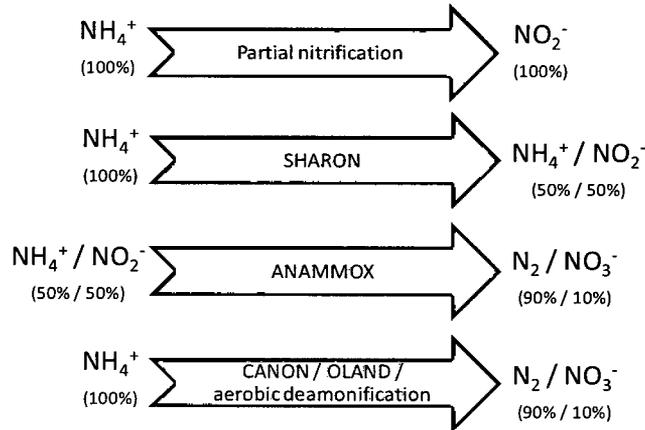


Figure 1.4 Schematic diagrams of partial nitrification, SHARON, Anammox, CANON, OLAND and aerobic deamonification processes. In brackets: percentages of nitrogen compounds in the flow (After Jubany, 2007).

Partial Nitrification

Partial nitrification, also known as nitritation is the oxidation of ammonium to nitrite by the AOB, while the oxidation of nitrite to nitrate carried out by NOB must be prevented. In this case, the further denitrification of the produced nitrite to nitrogen gas could be performed under autotrophic or heterotrophic conditions. Such combined biological nitrogen systems are partial nitrification-heterotrophic denitrification or partial nitrification-Anammox. In this way, several advantages with respect to the complete nitrification could be attained (Turk and Mavinic, 1987; Peng and Zhu, 2006): i) 40% reduction in COD requirements during denitrification; ii) 63% higher rate of denitrification; iii) 300% lower biomass production during anoxic growth; iv) 25% save of the oxygen requirements for nitrification due to suppression of the nitratation (oxidation of nitrite to nitrate) and v) 20% reduction of CO_2 emissions due to the denitrification from nitrite instead of nitrate. One example of successful partial nitrification system is the SHARON process developed in 1997 (Hellings et al., 1998). This process is carried out in a conventional continuous stirred tank reactor (CSTR) with suspended biomass and no sludge retention. At elevated temperature (30 and 40 °C) and low sludge retention time (SRT), AOB are selectively retained while the slow growing NOB, with growth rates lower than AOB at these conditions, are washed out from the system. Both nitrification and denitrification take place in the CSTR using intermittent aeration. Nitrogen removal efficiency close to 100% can be achieved.

The most critical condition that is needed for the success of partial nitrification process is to suppress nitrite oxidation without excessively retarding ammonium oxidation rate. Generation and maintenance of nitritation reactor requires that the NOB to be washed out from the system, or suppress suitable conditions to re-establish NOB growth (Sinha and