Universitat Autònoma de Barcelona

Departament d'Enginyeria Química
Escola d'Enginyeria

COMPARATIVE STUDY ON THE TREATMENT OF A HIGH-STRENGHT *p*-NITROPHENOL WASTEWATER

PhD Thesis

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Comparative study on the treatment of a high-strenght *p*-nitrophenol wastewater

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CERTIFIQUEM:

Que l'enginyera química MARIÁNGEL MARTÍN HERNÁNDEZ ha realitzat sota la nostra direcció el treball amb títol "Comparative study on the treatment of a high-strenght *p*-nitrophenol wastewater", que es presenta en aquesta memòria, i que constitueix la seva tesi per a optar al Grau de Doctor per la Universitat Autònoma de Barcelona.

I perquè en prengueu coneixement i consti als efectes oportuns, presentem a l'Escola d'Enginyeria de la Universitat Autònoma de Barcelona l'esmentada tesi, signant el present certificat a Bellaterra, Juliol 2012.

Dr. Julián Carrera

Dra. María Eugenia Suárez Ojeda

I dedicate this thesis to my parents Miguel and Maribel,
to my sisters Carmen Nieves and Ariadna
and to my boyfriend Jose Manuel.

“A person who never made a mistake never tried anything new”
Albert Einstein

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Summary

p-Nitrophenol (PNP) is an important compound for the chemical industry due to its numerous applications. PNP is used mainly as raw material in the manufacturing industry of pharmaceutical products and pesticides. It is also used for manufacturing leather-related products. Despite its wide use, PNP is highly toxic for humans, animals and the environment. Repeated exposure to PNP might cause injury to blood cells (methemoglobinemia), damage to the central nervous system and mutagenic effects.

In spite of all physical, biological and chemical processes available to treat wastewaters, it is still a difficult task to select one single treatment for recalcitrant compounds, such as PNP, that are frequently present in industrial wastewaters. The selection of the appropriate process not only depends on the nature of the recalcitrant compound but also on its concentration and wastewater-loading rate.

Therefore, in this thesis a comparative study on the treatment of high-strength wastewaters containing PNP is intended for its removal from a model industrial wastewater by taking different approaches according to the wastewater characteristics.

Firstly, biological treatment in an aerobic Sequencing Batch Reactor (SBR) was performed. The start up of the reactor was done using a non-acclimated biomass coming from a municipal wastewater treatment plant (WWTP) as inoculum and a synthetic wastewater containing a mixture of PNP and glucose-sucrose as carbon sources. A specific operational strategy was applied with the main aim of developing a K-strategist PNP-degrading activated sludge and following a feeding strategy in which the PNP-degrading biomass was under endogenous conditions during more than 50% of the aerobic reaction phase.

In this case, hundred per cent of PNP removal was achieved over the course of the entire operating period with a maximum specific PNP loading rate of 0.26 g PNP g^{-1} VSS d^{-1}. A kinetic characterization of the obtained PNP-degrading population was carried out using respirometry assays. With the experimental data obtained, a kinetic model including substrate inhibition

was used to describe simultaneously the time-course of the PNP concentration and specific oxygen uptake rate (SOUR). The values obtained for the K_S and k_{max} were lower than those reported in the literature for mixed populations, meaning that the biomass was a K-strategist type, and therefore demonstrating the success of the operational strategy imposed to obtain such a K-strategist population. Moreover, our measured K_i value was higher than those reported by most of the bibliographic references; therefore the acclimated activated sludge used in this work was evidently more adapted to PNP inhibition than other reported cultures.

Afterwards, bioaugmentation using the developed PNP-degraders population was applied as a technology for facing transient or continuous shock loads of PNP, which is the most common scenario for industries effluents, where the production is not continuous. The effect of the amount of the enriched microbial population added for bioaugmentation was assessed by using two different dosages (2 or 5% w/w of the total biomass in the seeded SBR). In both cases, total PNP removal was achieved during the transient PNP shock load occurring after bioaugmenting the SBR. However, after a long PNP starvation period, the only experiment still showing total PNP removal during a second PNP shock load was the one where a dosage of 5% w/w was applied. Moreover, fluorescence in-situ hybridization (FISH) was used to follow the microbial population evolution during experiments in order to explain the obtained results. Results suggested that the dosage of specialized bacteria is the key factor for the implementation of a successful bioaugmentation strategy. In addition, the start-up performance of a bioaugmented SBR receiving a continuous PNP shock load was enhanced when compared to a non-bioaugmented SBR.

Finally, a different approach was taken in the case of highly concentrated PNP wastewaters that cannot be degraded in a biological reactor. So that, wet air oxidation (WAO) and catalytic wet air oxidation (CWAO) were performed to oxidize a highly concentrated PNP wastewater, aiming to increase the biodegradability prior to a biological remediation. In this sense, the influence of temperature, oxygen partial pressure, type of catalyst, pH and ionic strength on PNP CWAO was studied. Batch tests were performed and four Pt

and Ru-based catalysts were tested. PNP elimination, total organic carbon (TOC) abatement and the intermediates distribution were monitored. Respirometric screening tests were completed after each experiment to assess the biodegradability enhancement of the CWAO treated effluents. The results showed that PNP elimination was higher than 90% in most cases, being the temperature the most important operating parameter upon CWAO. Additionally, all the catalysts showed a similar behavior in terms of PNP and TOC conversions. Besides, CWAO increased the biodegradability by more than 50% in most of the tested conditions, being the carboxylic acid fraction the key factor to be taken into account, as the best biodegradability enhancement was observed when this fraction was the highest. On the contrary, partial pressure of oxygen had a negligible effect on the biodegradability enhancement. The ionic strength influence over the CWAO was studied and even though it did not affect the CWAO performance, the presence of NaCl in the solution resulted in a decrease of the effluent biodegradability. In terms of pH, the most suitable scenario was the one with no pH adjustment. Conclusively, it was determined that an integrated CWAO and biological treatment would allow an easy removal of highly concentrated PNP and the intermediates formed during the first step of the treatment.

In conclusion, this research contributed to a deeper understanding of the dynamics of the degradation of PNP under different technologies and provides an aid on the decision making process regarding the proposal of a best available technology for industrial wastewater treatment.

Resumen

El *p*-Nitrophenol (PNP) es un compuesto importante dentro de la industria química debido a la gran cantidad de aplicaciones en las que es utilizado. El PNP es principalmente empleado como materia prima en la industria farmacéutica y de pesticidas, así como también en la industria manufacturera del cuero. A pesar de ser un compuesto ampliamente usado, el PNP es extremadamente tóxico para los humanos, animales y el medio ambiente. Exposiciones repetidas a este compuesto pueden causar daños a los glóbulos rojos, ocasionando una enfermedad llamada metahemoglobinemia y también puede ocasionar daños en el sistema nervioso central y producir efectos mutagénicos.

Proponer un tratamiento único para eliminar los compuestos recalcitrantes que se encuentran presentes en las aguas residuales industriales, como es el caso del PNP, no es una tarea sencilla a pesar de que se dispone de numerosos tratamientos físicos, biológicos y químicos. Es así como la selección de un proceso de tratamiento adecuado depende no solo de la naturaleza del compuesto a eliminar sino también de la concentración del mismo y de la carga a tratar.

Por consiguiente, en esta tesis se realizó un estudio comparativo de diversas tecnologías de tratamiento para la remediación de aguas residuales con una alta concentración de PNP con el objetivo de conseguir una su eliminación óptima. Para ello, se aplicaron distintos enfoques de acuerdo a las características del agua residual.

En primer lugar se estudió el tratamiento biológico de aguas contaminadas con PNP en un reactor aeróbico secuencial por lotes (SBR, por sus siglas en inglés). La puesta en marcha del reactor fue realizada utilizando como inóculo biomasa no aclimatada proveniente de una estación depuradora de aguas urbanas (EDAR) y agua residual sintética que contenía una mezcla de PNP y glucosa–sacarosa como fuentes de carbono. Se aplicó una estrategia operacional específica con el objetivo principal de desarrollar lodos activos del tipo estrategas de la K capaces de degradar PNP y de soportar condiciones de hambruna. En esta estrategia la biomasa

degradadora de PNP estuvo bajo condiciones endógenas durante más del 50% de la fase aeróbica de reacción.

Durante todo el período de operación se obtuvo un 100% de eliminación de PNP, y se logró trabajar con una carga máxima específica de PNP de 0.26 g PNP g^{-1} SSV d^{-1}. Se llevaron a cabo estudios respirométricos para realizar una caracterización cinética de la población degradadora de PNP. Con los datos experimentales obtenidos, se aplicó un modelo cinético que incluía la inhibición por sustrato para describir simultáneamente la degradación del PNP y la tasa específica de consumo de oxígeno (SOUR, por sus siglas en inglés). Los valores obtenidos para K_s y k_{max} fueron inferiores a los reportados en la bibliografía para poblaciones mixtas, lo cual significa que la biomasa obtenida era del tipo estrategas de la K, demostrando, por lo tanto, el éxito de la estrategia operacional impuesta para obtener este tipo de población. El valor de K_i que se obtuvo resultó ser mayor a otros reportados en la bibliografía, por lo que se dedujo que la población obtenida en este estudio estaba más adaptada a la inhibición por PNP que otros cultivos reportados previamente.

En segundo lugar, se empleó la población desarrollada previamente para realizar la bioaumentación de un SBR con el objetivo de hacer frente a choques de carga de PNP transitorios o continuos, que son los escenarios más frecuentemente encontrados en los efluentes industriales. En este caso se estudió el efecto de la cantidad de biomasa añadida en la bioaumentación utilizando dos dosis diferentes (2 o 5% p/p del total de la biomasa en el reactor bioaumentado). En ambos casos, luego de bioaumentar el SBR, se consiguió la eliminación total del PNP durante los choques de carga transitorios. Sin embargo, luego de un largo período de hambruna, se obtuvo eliminación total de PNP solo en el caso en donde fue empleada una dosis de 5% p/p. Por otra parte, se monitorizó la evolución de la población microbiana durante los experimentos de bioaumentación mediante hibridación fluorescente in situ (FISH, por sus siglas en inglés) con el objetivo de explicar los resultados obtenidos. Los resultados sugirieron que la dosis de microorganismos especializados empleada en la bioaumentación es el factor clave para la implementación exitosa de esta estrategia de tratamiento.

Asimismo, la puesta en marcha de un SBR que recibía choques de carga continuos de PNP mejoró sustancialmente al utilizarse la bioaumentación en comparación con la puesta en marcha del mismo SBR no bioaumentado.

Finalmente, se tomó un enfoque diferente en el caso de aguas residuales altamente concentradas en PNP y que no pueden ser degradadas en un reactor biológico. Así pues, se ensayaron la oxidación húmeda con aire (WAO por sus siglas en inglés) y la oxidación húmeda catalítica con aire (CWAO por sus siglas en inglés) para el tratamiento de aguas residuales altamente concentradas en PNP con el objetivo de incrementar la biodegradabilidad de dichos efluentes. En este sentido, se estudiaron la influencia de la temperatura, de la presión parcial de oxígeno, del tipo de catalizador empleado, del pH y de la fuerza iónica. Se realizaron experimentos en discontinuo se emplearon cuatro catalizadores soportados de Pt y Ru. Se monitorizaron la eliminación del PNP, del carbono orgánico total (COT) y la distribución de los intermediarios de reacción.

Por otra parte, se realizaron ensayos respirométricos para valorar la mejora en la biodegradabilidad de los efluentes tratados por CWAO. Los resultados muestran que la eliminación de PNP fue superior al 90% en la mayoría de los casos, siendo la temperatura el factor más importante. Adicionalmente, todos los catalizadores mostraron un comportamiento similar en términos de conversión de PNP y de COT. La mejora de la biodegradabilidad de los efluentes fue superior al 50% en la mayoría de los casos, siendo la fracción de ácidos carboxílicos el factor clave a ser tenido en cuenta, puesto que la mejora en la biodegradabilidad era directamente proporcional al aumento de la fracción de ácidos carboxílicos. Por el contrario, la presión parcial de oxígeno tuvo un efecto poco significativo en la mejora de la biodegradabilidad, mientras que la presencia de NaCl resultó en una disminución de la biodegradabilidad de los efluentes, aunque no causó ningún efecto adverso sobre la etapa de oxidación. En términos de pH, el mejor escenario fue aquel donde no se requiere ajuste del mismo. En conclusión, se determinó que la integración de CWAO con un tratamiento biológico permitiría una eliminación completa del PNP y de los intermedios que se forman durante la etapa de oxidación.

En conclusión, esta tesis contribuye a una comprensión más profunda de la dinámica de degradación del PNP bajo diferentes tecnologías y provee de una ayuda en el proceso de toma de decisiones en cuanto a la selección de la mejor tecnología disponible para el tratamiento de aguas residuales industriales.

Abbreviation list

- 1,2,4-BT: 1,2,4-benzenetriol
- 4-NC: 4-nitrocatechol
- 2,4-DNP: 2,4-dinitrophenol
- α: Confidence level
- σ: Standard error
- $\vec{\theta}$: Vector of model par
- *C*: covariance matrix
- CLSM: Confocal laser scanning microscopy
- CWAO: Catalytic wet air oxidation
- DO: Dissolved oxygen
- E-PRTR: European Pollutant Release and Transfer Register
- FIM: Fisher information matrix
- FISH: Fluorescent in situ hybridization
- HPLC: High performance liquid chromatograph
- HQ: Hydroquinone
- HRT: Hydraulic retention time
- LFS: Liquid-flow-static
- *G*: Objective function in equation 3.5
- *J*: Objective function in equation 3.6
- k_{max}: Maximum specific PNP removal rate
- K_s: Half-saturation coefficient for PNP
- K_i: Substrate inhibition constant for PNP
- *N*: Number of data points
- OC: Oxygen consumption
- OUR: Oxygen uptake rate
- *p*: Number of parameters
- *p*-BQ: *p*-benzoquinone
- PAP: *p*-aminophenol
- PFOA: Perfluorooctanoic acid
- PFOS: Perfluorooctanesulfonic acid

- PNP: *p*-nitrophenol
- *s*: Residual mean
- SBR: Sequencing batch reactor
- SOUR: Specific oxygen uptake rate
- SRT: Sludge retention time
- TSS: Total suspended solids
- TOC: Total organic carbon
- VSS: Volatiles suspended solids
- $\vec{w}$: Vector of weighting coefficients in equation 3.6
- WAO: Wet air oxidation
- WWTP: Wastewater treatment plant
- *X*: Total biomass concentration
- $\vec{y}$: Vector of either experimental data or modeling results depending on the superscript used
- $Y_{X/PNP}$: Yield coefficient for PNP biodegradation

CHAPTER 1

General introduction

1.1. Water pollution by industrial effluents

Water is the natural resource from which our economic and social activities depend on; its scarce nature and the fast growing economies make imperative to address water issues from a technical, management, social and political point of view. The urban population in 2009 was 3.4 billion and predictions say that it will grow to over 6.3 billion people in 2050, this includes the normal expected growth and some net population movement from rural areas to urban ones with the inherent requirements of adequate water supply, sanitation, drainage and wastewater treatment (UNESCO, 2012).

Basically, all the activities that support our current lifestyle, namely activities that generate goods and services, produce contaminants; consequently, there is a great pressure over water resources from manufacturing industry and this is due to the impact of its wastewater discharges and their contaminating nature, rather than the water directly used in production process. The most important water contaminants generated by the industry are nutrients, oxygen consuming pollutants, suspended sediments, heavy metals and persistent or toxic organic matter.

Water pollution is increasing around the world as a consequence of inadequate disposal of contaminated water. Treating wastewaters is essential to prevent environmental pollution and to preserve public health by protecting the water supplies and preventing the spread of water-borne diseases. When industrial wastewater is discharged without treatment to open watercourses, the quality of a great volume of water is reduced and may contaminate recreational water bodies and groundwater resources. According to the United Nations – World Water Assessment Program, over 80% of used water in the world is not collected or treated prior to discharge polluting rivers, lakes and

coastal areas, therefore, compromising fresh water sources (UNESCO, 2012).

Understanding water demand is mandatory to make a proper assessment on water management and effluent treatment. On this matter, around 70% of the fresh water used in the world is intended for agriculture use, being next the industrial use with 19% and municipal use with 11%. At global scale, though these numbers considerably vary between regions and countries, marking high differences between developed and developing countries. It is estimated that industry in low-income countries uses around 5% of fresh water and this value increases up to 40% for high-income countries (UNESCO, 2012). Details on the distribution of fresh water usage by sector, including desalinated water, are shown on Table 1.1.

Although the industry requirement of water is relatively little on a global scale, it demands an accessible, reliable and environmentally sustainable supply. In this sense, industrial pollution is expected to increase in developing countries where their emerging market economy and industrial development have attracted many industries, especially of the chemical, leather and organic raw materials to move their operations there, where the access to wastewater treatment is limited. In this sense, lot of pressure and expectations are posed over this industry to address their wastewater problem.

Pollutants may enter receiving waters by diffuse sources (non-specific point source of discharge). The major source of pollution would be agriculture, but in urban areas atmospheric deposition by runoff may also be an important source; also, pollutants can enter by the point sources that are the discharges from the urban wastewater treatment facilities. It is unusual for modern urban wastewater treatment plants (WWTP) to receive wastewater from industrial complexes, such as chemical, manufacturing, brewing, meat processing, metal processing or paper mills unless the treatment plant is specifically designed to do it. In developed countries most industries treat their own wastewaters to reduce the pollutant load and to meet discharge limits imposed by law before the public sewer.

Table 1.1. Water withdrawal by sector (FAO, 2010).

Continent Regions	Sub regions	Total withdrawal by sector					
		Municipal		Industrial		Agricultural	
		km^3/year	%	km^3/year	%	km^3/year	%
World		*429*	*11*	*723*	*19*	*2710*	*70*
Africa		*21*	*10*	*9*	*4*	*184*	*86*
	Northern Africa	9	9	5	6	80	85
	Sub-Saharan Africa	13	10	4	3	105	87
Americas		*126*	*16*	*280*	*35*	*385*	*49*
	Northern America	88	15	256	43	258	43
	Central America and Caribbean	6	26	2	11	15	64
	Southern America	32	19	21	13	112	68
Asia		*217*	*9*	*227*	*9*	*2012*	*82*
	Middle East	25	9	20	7	227	83
	Central Asia	5	3	8	5	150	92
	Southern and Eastern Asia	186	9	200	10	1635	81
Europe		*61*	*16*	*204*	*55*	*109*	*29*
	Western and Central Europe	42	16	149	56	75	28
	Eastern Europe	19	18	56	51	35	32
Oceania		*5*	*17*	*3*	*10*	*19*	*73*
	Australia and New Zealand	5	17	3	10	19	73
	Other Pacific Islands	0.01	14	0.01	14	0.05	71

The objective of wastewater treatment is to allow effluents to be discharged without endangering human health and without damaging the environment. In that matter and regarding industrial emissions, in Europe, the Council Directive concerning urban wastewater treatment (91/271/EEC) states that the industrial wastewater entering collecting systems and urban wastewater treatment plants shall be subject to a pre-treatment in order to (i) protect the health of staff working in collecting systems and treatment plants,

(ii) ensure that collecting systems, wastewater treatment plants and associated equipment are not damaged, (iii) ensure that the operation of the wastewater treatment plant and the treatment of sludge are not impeded, (iv) ensure that discharges from the treatment plants do not adversely affect the environment or prevent receiving water from complying with other community directives and (v) ensure that sludge can be disposed of safety in an environmentally acceptable manner.

In addition, the European Directive on industrial emissions (2010/75/EU) declares that for each member state, the competent authorities shall establish the limit values for emissions for the chemical industry; the directive also requires the use of the best available technique and environmental quality standards for emissions treatment. The same directive establishes which industries are permitted to directly discharge into urban WWTP and the industries that are required to treat their effluents before disposal. This means that a great deal of organic compounds that might not be biodegradable could enter the sewer system and if the urban WWTP cannot deal properly with the situation, it might cause that a large sum of pollutants end up discharged directly into receiving waters (river basins, lakes, oceans).

In this work, the degradation of *p*-nitrophenol (PNP) is studied. In Europe, the information of releases and transfers for all phenolic compounds are grouped and reported as kg of carbon. Table 1.2 shows the situation of phenolic compound releases for the European continent, where most of the releases come from power stations and urban WWTP. In this particular case, this means that the urban WWTP do not have the capacity to treat phenolic compounds that could enter the sewer system by seasonal runoff or by industry discharges. In Spain, the major source for phenolic compound releases comes from the steel or pig iron production or from refineries (Table 1.3).

Table 1.2. Phenolic compounds releases (expressed as kg of carbon) to receiving waters in the 27 members of the EU and Iceland, Liechtenstein, Norway, Serbia and Switzerland (E-PRTR, 2012).

Industrial Activity	**Total discharges (kg C)**	**%**
Thermal power station and other combustion installations	652,591.9	63.5
Urban wastewater treatment plants	195,473.0	19.0
Mineral oil and gas refineries	73,239.1	7.1
Industrial scale production of basic organic chemicals	24,108.8	2.4
Production of pig iron or steel including continuous casting	21,409.2	2.1
Metal ore (including sulphide ore) washing or sintering installation	11,699.1	1.1
Production of pulp from timber or similar fibrous materials	9,508.1	0.9
Independently operated industrial waste water treatment plants serving a listed activity	9,144.8	0.9
Industrial scale production of basic inorganic chemicals	8,735.0	0.9
Other	21,445.1	2.1
Total	*1,027,354.1*	*100.0*

Table 1.3. Phenolic compounds releases (expressed as kg of carbon) to receiving waters in Spain (E-PRTR, 2012).

Industrial Activity	**Total discharges (kg C)**	**%**
Production of pig iron or steel including continuous casting	9,380.0	50.4
Mineral oil and gas refineries	4,213.8	22.6
Production of non-ferrous crude metals from ore, concentrate or secondary raw materials	3,650.0	19.6
Industrial scale production of basic organic chemicals	700.1	3.7
Treatment and processing of animal and vegetable materials in food and drink production	404.0	2.2
Industrial scale production of basic pharmaceutical products	151.0	0.8
Disposal of non-hazardous waste	65.5	0.4
Production of paper and other primary wood products	56.1	0.3
Incineration of non-hazardous waste included in directive 2000/76/EC waste incineration	53.6	0.3
Landfills (excluding landfills closed before the 16/7/2001)	24.4	0.1
Total	*18,620.5*	*100.0*

For phenolic compounds, the greater amount of transfers corresponds to the basic organic chemicals industry, mineral and oil and coke ovens (Table 1.4). In the case of Spain, the industry of pyrotechnics and explosives production is the biggest contributor to phenol transfers (Table 1.5). Comparing releases and transfers in Europe, the register indicates that the net amount of transfers is higher than the releases to watercourses, but in Spain the situation is opposite, this indicates that there is still a lot of work that needs to be done to prevent water contamination.

Table 1.4. Phenolic compounds transfers (expressed as kg of carbon) in the 27 members of the EU and Iceland, Liechtenstein, Norway, Serbia and Switzerland (E-PRTR, 2012).

Industrial Activity	**Total discharges (kg C)**	**%**
Coke ovens	351,734.0	28.7
Industrial scale production of basic organic chemicals	330,086.0	27.0
Production of pig iron or steel including continuous casting	105,720.0	8.6
Mineral oil and gas refineries	105,543.0	8.6
Industrial scale production of basic pharmaceutical products	105,364.0	8.6
Processing of ferrous metals	72,517.0	5.9
Disposal or recovery of hazardous waste	52,528.7	4.3
Landfills (excluding landfills closed before the 16/7/2001)	50,147.3	4.1
Disposal of non-hazardous waste	9,312.5	0.8
Other	41,305.3	3.4
Total	*1,224,257.8*	*100.0*

Table 1.5. Phenolic compound transfers (expressed as kg C) in Spain (E-PRTR, 2012).

Industrial Activity	Total discharges (kg C)	%
Industrial scale production of explosives and pyrothecnic products	1,520.0	26.1
Pre-treatment or dying of fibers or textiles	991.5	17.0
Landfills (excluding landfills closed before 16/7/2001)	850.5	14.6
Mineral oil and gas refining	397.0	6.8
Industrial scale production of basic organic chemicals	345.5	5.9
Production of pig iron or steel including continuous casting	299.8	5.1
Surface treatment of substances, objects or products	255.7	4.4
Production of paper and board and other primary wood products	223.0	3.8
Treatment and processing of animal and vegetable materials in food and drink production	201.0	3.5
Other	743.0	12.8
Total	*5,827.0*	*100.0*

1.2. Model compound selected: *p*-nitrophenol

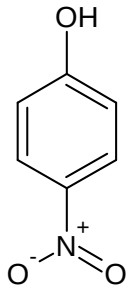
To select the model compound for this study, several aspects were taken in consideration. PNP is one of the most used nitrophenolic compounds, it is included in the High Volume Production Chemicals by the Organization for Economic Cooperation and Development which means that is produced in quantities higher than 1,000 ton/year in at least one member/country (OECD, 2008). Last report on its demand in the US said that in 1995, 11,500 ton of PNP were used in the country; its major uses include (NLM, 1992):

- The pharmaceutical industry as a raw material in the manufacture of paracetamol (acetaminophen).
- The manufacture of ethyl and methyl parathion, an insecticide and acaricide used for pest control in agriculture that nowadays its use is forbidden or restricted in several countries. The molecule of PNP appears as a metabolite on parathion degradation.
- As a fungicide.
- As a leather tanning agent.
- As pH indicator for chemical analysis.

- As a pesticide itself.

Despite a wide range of uses, PNP is highly toxic for humans and for the environment; human exposure may come primarily through impurities in pharmaceuticals and polluted water and food. Even though there is no conclusive information on its effects on humans, its effects in rats show that PNP may cause injury to blood cells (methemoglobinemia), damage to the central nervous system and mutagenic effect. No information about if PNP can produce cancer is known (ATSDR, 1992; Eichenbaum et al., 2009). Chemical and physical properties of PNP are summarized in Table 1.6.

Table 1.6. Chemical and physical properties of PNP (Sigma-Aldrich, 2012).

Chemical name	4-nitrophenol 4-hidroxynitrobenzene *p*-nitrophenol
Chemical formula	$C_6H_5NO_3$
Chemical structure	
CAS number	100-02-7
Molecular weight	139.11 g mol^{-1}
Colour	Light yellow
Appearance form	Crystalline
Melting point	110-115ºC
Boiling point	279ºC
Flash point	169ºC - closed cup
Vapour pressure	9.2 hPa at 165ºC - 0.8 hPa at 120ºC
Relative density	1.48 at 20ºC
Water solubility	15 g/l
Auto ignition temperature	283ºC

A study carried out on the quality of European rivers, where more than 100 river water samples from 27 European countries were analyzed for selected organic compounds including pharmaceuticals, pesticides, perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), benzotriazoles and hormone and endocrine disrupters concluded that only 10% of those river samples could be classified as very clean in terms of chemical pollution, identifying as well the most polluted rivers (Loos et al., 2009) PNP was one of the most frequent compounds found in the rivers being on 97% of the analysed samples (Figure 1.1) with a maximum concentration of 3471 ng L^{-1} and an average of 99 ng L^{-1}. These findings permit a wider assessment on the environmental risks and the necessities for treatment.

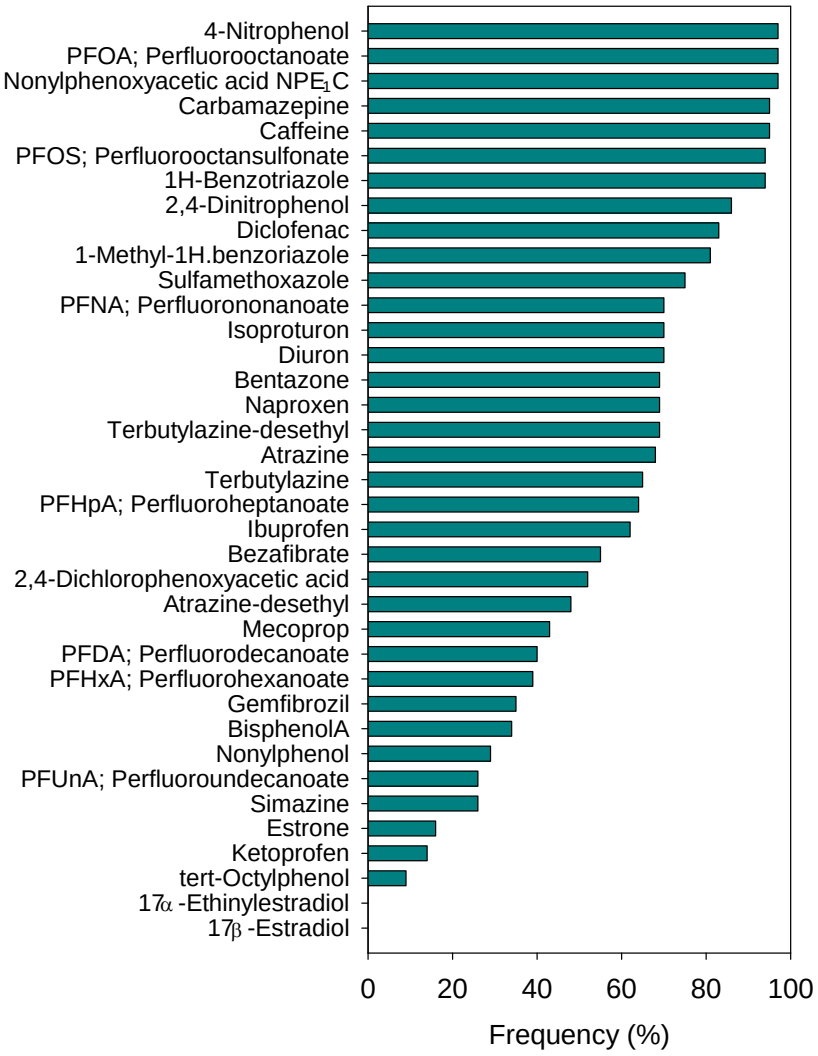


Figure 1.1. Summary of pollutants present in the EU rivers represented as the frequency of appearance in tested samples (Loos et al., 2009)

In Europe, the releases to water bodies and transfers to waste management of PNP are tabulated within the total phenolic compound discharges and its absolute value is not publicly reported (Table 1.2 and Table 1.4). In Japan, the annual report on total releases and transfers includes PNP on its list. In the last report, 290 kg $year^{-1}$ of PNP were released to water bodies while 24,022 kg $year^{-1}$ were transferred to waste treatment facilities (NITE, 2007). In United States, the Toxic Release Inventory has reported for 2010 a total of PNP releases of 108 kg and 179 kg of transfers to waste management (TRI, 2012). In the United States, PNP is listed as a priority pollutant by the United States Environment Protection Agency (EPA, 2010) and the releases and transfers from chemical industry are controlled by the Code of Federal Regulation, establishing a limit of 124 $\mu g\ L^{-1}$ as maximum per day or 72 $\mu g\ L^{-1}$ as a month average for the PNP discharges from point sources that use end of pipe biological treatment and of 576 $\mu g\ L^{-1}$ as maximum per day and a maximum monthly average of 162 $\mu g\ L^{-1}$ for facilities that do not use end of pipe biological treatment to comply with the regulation is (Protection of Environment, 2010).

1.3. Available process to treat wastewater polluted with PNP

The selection of a treatment process is dependent on the nature of the wastewater and the desired quality of the effluent. Hazardous components like PNP may be either separated or converted to non-hazardous forms, in order to permit the disposal of the wastewater effluent. In the case of PNP degradation there are several treatments available. In the following sections, available techniques to degrade PNP are briefly described and the state of the art of the most relevant processes to degrade PNP containing wastewaters is presented.

1.3.1. Biological treatments: anaerobic or aerobic treatment of PNP

In the remediation of wastewaters, biological treatment attracts the attention of industrial sector because is a process relatively less expensive

than chemical treatments. With the proper environmental conditions, seed source and acclimation time, a wide range of toxic and recalcitrant organic compounds have been found to be biodegradable. In the microbial transformation of nitro-aromatics compounds, both aerobic and anaerobic processes can be used; in the aerobic process microorganisms use the dissolved oxygen on the liquid to convert the organic wastes to CO_2 and biomass while in the anaerobic process, microorganisms convert the organic wastes into methane, CO_2 and H_2O in the absence of oxygen. Currently, the investigation on microbial remediation is focused on improvement on the knowledge of the catabolic pathways of degradation and the optimization of parameters to accelerate the degradation (Kulkarni and Chaudhari, 2007).

The ability to degrade recalcitrant compounds like PNP will depend primarily on the presence of appropriate microorganisms and the acclimation performed. In this sense, lots of effort have been made in isolating strains capable of use PNP as carbon source and describing the degradation pathways (Crawford, 1995; Spain and Gibson, 1991). The biodegradation of PNP in pure cultures has been investigated in species like *Bacilllus sphaericus* (Kadiyala et al., 1998), *Pseudomonas putida* (Kulkarni and Chaudhari, 2007; Löser et al., 1998), *Moraxella* sp (Spain and Gibson, 1991), *Arthrobacter* sp (Jain et al., 1994), *Acinetobacter* and *Arthrobacter* sp (mixed culture) (Suárez-Ojeda et al., 2011) that were able to degrade PNP.

The decision between aerobic and anaerobic treatment is not always an easy task. The advantages of anaerobic treatment outweigh the aerobic treatment when treating influents of high concentrations and generally, it requires less energy with the potential of energy recovery. But, aerobic systems provide higher efficiencies on organic removal. Several features of aerobic and anaerobic treatment for industrial and municipal wastewaters are compared and presented on Table 1.7.

In general, aerobic systems are suitable for the treatment for wastewater with biodegradable concentrations of chemical oxygen demand (COD) less than 1000 mg L^{-1} and anaerobic systems are suitable for the treatment of wastewaters with biodegradable COD concentration over 4000 mg L^{-1}. But in most applications, despite the efficiency of the anaerobic process is high, the

final effluent produced do not necessarily meet the requirements for discharge, requiring a post-treatment with an aerobic reactor. For these industrial wastewaters, anaerobic-aerobic processes may present a high overall treatment efficiency (Chan et al., 2009).

Table 1.7. Comparison of aerobic and anaerobic treatment (Chan et al., 2009).

Feature	Aerobic	Anaerobic
Organic removal efficiency	High	High
Effluent quality	Excellent	Moderate to poor
Organic loading rate	Moderate	High
Sludge production	High	Low
Nutrient requirement	High	Low
Alkalinity requirement	Low	High for certain industrial waste
Energy requirement	High	Low to moderate
Temperature sensitivity	Low	High
Start up time	2-4 weeks	2-4 months
Odor	Less opportunity for odors	Potential odor problems
Bioenergy and nutrient recovery	No	Yes
Mode of treatment	Total (depending on feedstock characteristics)	Essentially pretreatment

In the case of anaerobic treatment of nitro-aromatics, several studies have demonstrated reduction of nitro groups to amino groups as a prelude to degradation, often leaving them as an undesired intermediate on the effluents (Kulkarni and Chaudhari, 2007; Razo-Flores et al., 1997).

Sponza and Kusçu (2005) have used a configuration of an anaerobic migrating blanket reactor (AMBR) combined with an aerobic continuous stirred tank reactor (CSTR) for simultaneous degradation of PNP and methane production. The configuration permitted to achieve efficiencies of 94 and 95% respectively with a stable production of methane; but the main problem with this configuration and with anaerobic treatment of PNP in general is that the PNP was transformed to phenol and *p*-aminophenol (PAP) in the anaerobic making the inlet of the CSTR of high complexity.

In the anaerobic treatment of PNP, the presence of a co-substrate is required for the microbial growth. Glucose is most commonly used with relatively high methane production efficiency; but also acetate and methanol might be employed. PAP is the major intermediate found in different reactor configurations (Kusçu and Sponza, 2009; Karim and Gupta, 2002).

The inhibition of the anaerobic process to biodegrade PNP and the toxic intermediates produced had pushed the investigation towards the biodegradation by aerobic bacteria.

In natural environments, the recalcitrant compounds are usually breakdown into other molecules by a microbial consortia rather than a single strain (Crawford, 1995), nevertheless, there are cases of successful operation on PNP degradation using single strain; in a study where *Arthrobacter chlorophenolicus A6* was used to inoculate an up-flow packed bed reactor successfully degraded high loads of PNP as a sole carbon source with a stable operation of more than 100 days (Sahoo et al., 2011). However, from a practical point of view, the operation in a full-scale aerobic PNP biodegradation plant with isolated strains would be difficult. Accordingly, several studies have obtained PNP-degrading populations from activated sludge (Bhatti et al., 2002; Rezouga et al., 2009; Salehi et al., 2011; Tomei et al., 2003; Xing et al., 1999; Yi et al., 2006) with different configurations and achieving stable operations for more than 100 days in most of the cases. The kinetic degradation from the aerobic PNP degradation had also been widely studied with authors reporting a large variability in the estimated parameters. Rezouga et al. (2009) explains that this difference could be due to the variability in the biomass history, changes in culture conditions (food to microorganism ratio, dilution rate, presence or lack of a co-substrate lead to various metabolic states as well as a different number of acclimated organisms in the overall population.

Most of the studies coincide in the need of a readily co-substrate in the feed along with PNP to improve the microbial growth and to increase the PNP degradation rate being glucose the preferred co-substrate for reactors working with pure or mixed culture (Bhatti et al., 2002; Qiu et al., 2006; Salehi et al., 2011; Yi et al., 2006). Other studies have used successfully propionate and

ethanol as a co-substrate for carbon source (Rezouga et al., 2009) or even in a reactor using granules, hydroquinone (HQ), 4-nitrocatechol (4NC) and PAP, finding that the PNP-degrading granules can use HQ and 4NC as carbon source but not PAP (Yi et al., 2006).

Substrate inhibition is one of the most frequent problems for PNP degradation, which is frequently reported (Kadiyala et al., 1998; Ray et al., 1999; Tomei et al., 2003) although Yi et al (2006) could develop a biomass significantly less susceptible to the effects of substrate inhibition by working with aerobic granules. Finally, the oxygen supply strongly affects the PNP aerobic biodegradation as demonstrated by several studies (Ray et al., 1999; Sahoo et al., 2011; Salehi et al., 2011). In this thesis the objective was to obtain a microbial population with high affinity to degrade a high-strength PNP wastewater and less susceptible to substrate inhibition. This microbial population could be useful for bioaugmentation applications.

1.3.2. Bioaugmentation of an aerobic biological treatment

The degradation of a pollutant in a fixed environment (e.g. soil, wastewater or sediment) by a microbial population can be enhanced by either biostimulation of the indigenous bacteria (e.g. with the addition of nutrients or electron acceptors) or by bioaugmentation which is defined as the introduction of specific microorganisms, which could be specific strains or a microbial consortia, in the contaminated matrix in order to accelerate the pollutant removal of the environment (El Fantroussi and Agathos, 2005).

The inoculum survival has been considered a week point for bioaugmentation in contaminated land. Consequently, a lot of effort has been invested in strain selection to facilitate pollutant biodegradation; the most frequent method includes the isolation of specialized microorganisms able to resist high environmental stress, other approaches include harboring catabolically superior pollutant-degrading enzymes or the “priming” (Singer et al., 2005).

Priming is an approach that has been effectively demonstrated in environmental systems, where a portion of clean soil is enriched for pollutant

degrading microorganisms by repeated biostimulation with the relevant pollutant. This approach has several advantages, the inoculum is a consortium of indigenous microorganisms, which is potentially more resilient to stress than a single isolate, and the “primed” consortium is maintained within its native soil, potentially enhancing its survival in the target soil (Singer et al., 2005). In many cases, microbial consortia has been more effective than single strains by the fact that intermediates of a catabolic pathway of one strain may be further degraded by other strains (Mrozik and Piotrowska-Seget, 2010). This approach is expandable to be applied not only in soils but to wastewater as well, and the equivalent to the “priming” would be the acclimation of activated sludge.

Other factors influencing bioaugmentation include diffusional limitation of substrate supply, most evidenced in soil remediation than in wastewater treatment and probably the most important, the competition between indigenous and exogenous microorganisms for limited carbon sources, as well as, incompatible interactions and predation by protozoa and bacteriophages that potentially decrease the number of introduced cells (El Fantroussi and Agathos, 2005; Mrozik and Piotrowska-Seget, 2010; Vogel, 1996)

Despite all the potential of bioaugmentation, there is a lack on engineering design for bioaugmentation technique; in some studies the approach have been to add a high number of microorganisms to contaminated soils and thus, bioavailability would not be the limiting factor for bioaugmentation success (Vogel, 1996).

To the best of our knowledge, few studies have been conducted on bioaugmentation applications on wastewater or soil for PNP degradation. In a soil application, a slurry reactor was seeded with an activated sludge able to degrade PNP and it was demonstrated that the degradation could be enhanced considerably with the introduction of specialized bacteria. It was also observed that increasing the amount of specialized bacteria also increased PNP degradation rate (Wang et al., 2005). Another study for degradation of mixed wastewater composed of PNP and nitrobenzene had implemented bioaugmentation technique by doing it selectively, first adsorbing

PNP onto a resin to avoid the inhibitory effect of PNP over nitrobenzene degrading bacteria and then biodegrading PNP (Hu et al., 2008).

Most of the studies that have applied enriched activated sludge as the specialized microbial consortia in bioaugmentation have used larges doses of these specialized bacteria (25 to 32% w/w of the total biomass in the seeded reactor) to recover the reactor activity after shock loads (Chong et al., 1997; Jianlong et al., 2002; Quan et al., 2004). This is impractical for a scale-up to an industrial level. Bioaugmentation applications are not intended to only recover reactors after toxicant exposures or shock loads, but also to improve the start-up an the stability of biological reactors; in that sense, bioaugmentation with activated sludge have been implemented with success to improve the reactor performance. This investigation is then conducted to optimize the bioaugmentation operation to levels that could be implemented on industrial applications.

1.3.3. Chemical oxidation treatments available to degrade PNP

Chemical oxidation in wastewater treatment typically involves the use of oxidizing agents such as ozone, hydrogen peroxide, MnO_4, ClO_2, Cl_2, HOCL and O_2 to provoke a change in the chemical structure of a compound or a group of compounds. When chemical oxidation is used, it may not be necessary to completely oxidize a given compound or group of compounds. In many cases, partial oxidation is sufficient to render specific compounds more amenable to subsequent biological treatment or to reduce their toxicity. Since moderate to high concentration can be treated by biological systems, chemical oxidation processes are only advisable to wastewaters to high strength wastewaters; otherwise the running operation cost will not compensate the use of those processes.

1.3.3.1. Wet air oxidation (WAO) and catalytic wet air oxidation (CWAO)

Wet air oxidation (WAO) is a hydrothermal treatment process that has been commercialized for approximately 50 years, development of the technology took place in the United States and started to be commercialized

by the Zimpro Company in the early 1960s. Nowadays it counts with over 400 industrial or municipal WAO systems constructed (Levec and Pintar, 2007). The process operates in the sub-critical water temperature and pressure range with conditions usually under 320ºC and 214 bar. This process is usually applied for the treatment of high strength wastewaters with components that are difficult or uneconomical to treat via conventional biological treatment or incineration. WAO has also been used for the treatment of municipal sludge to minimize the consumption of landfill capacity. In the WAO processes, the organic contaminants dissolved in water are either partially degraded into biodegradable intermediates or mineralized into innocuous inorganic compounds such as CO_2. In the majority of WAO wastewater applications, subsequent biological treatment of the WAO effluent is required (Levec and Pintar, 2007).

Nevertheless, the WAO process could be excessively expensive if complete mineralization is required. Introduction of catalysts have permitted to reduce significantly the pressure and temperature required to oxidize the organic compounds, typically CWAO operates under oxygen pressure in a range of 5-200 bar at elevated temperatures 125-320ºC, and it is usually applied to effluents with chemical oxygen demand (COD) in the range of 5-10 g L^{-1}. Though it varies with the type of wastewater, the cost of CWAO is about half of WAO due to milder operating conditions and shorter residence time (Kim and Ihm, 2011).

Extensive literature could be found on the application of CWAO to industrial wastewaters. The results showed that the catalytic activity and stability depend mainly on the combination of metal and support, the preparation and pre-treatment method, the nature of pollutants and the reaction conditions. Most of the literature takes phenol as a model compound and is devoted to study new catalysts, kinetics, and reaction pathways, since is very frequent in industrial wastewater and is an intermediate of the oxidation pathway for most of the phenolic compounds. In the case of substituted phenols, the steric effect of the substituent affects the reactivity of the molecule under CWAO conditions, modifying the reaction pathways and the reaction rates (Collado et al., 2011). Few studies have been conducted on

PNP CWAO, the most representative studies are summarized in Table 1.8. From those studies, is evident the effect of temperature and pressure for PNP oxidation, Suárez-Ojeda et al. (2005) found no conversion in a continuous reactor at 140ºC and 2 bar O_2 using activated carbon as catalyst, Stüber et al (2005) found a slightly better result on conversion increasing the oxygen pressure, and Santos et al. (2006) obtained higher conversion by using a wider excess on oxygen pressure and using activated carbon as catalyst. The best result for PNP CWAO is the one reported by Pintar et al (1994), in this case 80% of PNP conversion was found with higher temperature and pressure than the rest of the studies presented on Table 1.8. Nonetheless, Liou et al (2010) obtained 100% of PNP conversion but using peroxide promoted CWAO. This thesis is exploring the use of noble metal based catalysts that have previously reported good results in the oxidation of 2-clorophenol (Li et al., 2007).

Table 1.8. Summary of studies on CWAO of PNP.

Catalyst	P (bar)	T (ºC)	[PNP] (g L^{-1})	Conversion (%)	Reference
Fe^{III} resin - Batch reactor	1 (H_2O_2 promoted)	80	0.5	100	(Liou et al., 2010)
AC- Continuous reactor	16 (O_2)	160	1.0	75	(Santos et al., 2006)
AC- Continuous reactor	2 (O_2)	140	5.0	0	(Suárez-Ojeda et al., 2005)
AC- Continuous reactor	9 (O_2)	140	5.0	9	(Stüber et al., 2005)
Cu/CeO2- Batch reactor	10	160	0.5	60	(Posada et al., 2006)
Comercial; Cu(9.3%wt.), Zn(6.9%wt.), Co(1.4%wt.)	30	190	3.0	50% at pH 5 80% at pH 12	(Pintar and Levec, 1994)

1.3.3.2. Other oxidation processes

Advanced oxidation processes (AOP) typically involve the generation and use of the hydroxyl radical (primarily but not exclusive) as a strong oxidant in a mechanism that destroy compounds that cannot be oxidized by conventional oxidants. Over the past 30 years, research and development

concerning AOPs include heterogeneous and homogeneous photocatalysis based on near ultraviolet (UV) or solar visible irradiation, electrolysis, ozonation, Fenton's reagent, ultrasound and WAO (Klavarioti et al., 2009).

AOPs are usually applied to low COD wastewaters because of the cost involved in the generation of the hydroxyl radical. Depending on the properties of the waste stream to be treated and the treatment objective itself, AOPs may be employed alone or as a pre-treatment stage to convert recalcitrant compounds into more readily biodegradable intermediates for a biological treatment. Several examples of AOPs oxidising PNP are presented next.

The PNP oxidation by a wet electrocatalytic oxidation process (WEO) in relatively mild conditions (T= 100ºC, PO_2= 4 times the theoretical oxygen demand (TOD), [PNP]= 1 g L^{-1}, PN_2= 5 bar, current density = 0-7.08 mA cm^{-2} and pH in the range of 2.7 – 10.6) (Dai et al., 2008). In that study, the reaction pathway was very similar to that reported for CWAO of PNP involving a free radical mechanism, and pH strongly affected the reaction rate.

A study on electro-Fenton method to degrade PNP was conducted with the aim of determine the kinetic parameters of reaction (Zhang et al., 2007). PNP solution was of 200 mg L^{-1} and different operation conditions were tested. The best result was obtained fixing a Fenton's reagent dosage Fe(III) to H_2O_2 molar ratio and feeding H_2O_2 continuously instead of doing it in one batch. Highest conversion reached was of 92% in the best conditions.

Sonophotocatalysis, which involves the combination of the ultrasonic irradiation, ultraviolet radiation and a semiconductor photocatalyst, which enhances the rate of chemical reactions by the formation of enhanced amounts of free radicals, have been studied to degrade PNP at a pilot scale (Mishra and Gogate, 2011). The concentration of PNP in the influent ranged between 10 and 100 mg L^{-1}. Even though the concentration of PNP treated can be managed by biological treatments, this study from the industrial point of view ads useful information in terms of successful implementation of sonophotocatalytic oxidation process into pilot scale.

1.4. Combination of chemical and biological treatments

Chemical oxidation for complete mineralization is generally expensive because the oxidation intermediates formed tend to be more resistant to their complete chemical degradation and integration of chemical and biological processes can provide economically viable and environmental friendly wastewater treatment options for removing pollutants from wastewater (Oller et al., 2011).

The combination of chemical and biological processes, whilst it often presents as an advantageous approach it has several difficulties that need to be taken in account: (i) selectivity toward the formation of intermediates molecules less biodegradable than the original, (ii) poor selection of the treatment conditions, leaving effluents with too little metabolic value for the microorganisms and (iii) the excess of catalyst used may result in traces of metal salts on the effluent that are normally toxic to microorganisms (Oller et al., 2011). As example of the application of WAO and CWAO as a precursor for biological treatment, Mantzavinos et al (1999) have found that the suitable wastewater pre-treatment conditions was significant for the effective application of an integrated process. Suárez-Ojeda et al. (2007a) have successfully implemented a combined CWAO - biological treatment to treat substituted phenols wastewater, the study was focused on determining if the effluents were suitable for a conventional activated sludge plant with non-acclimated biomass. Other studies from the same group have implemented successfully a coupled CWAO-biological treatment for *o*-cresol wastewaters (Suárez-Ojeda et al., 2007a). In the case of PNP, the studies of biodegradability of the effluent would be valuable to assess the feasibility of coupling the chemical with a biological treatment since the reaction pathway and the intermediates formed with different AOPs strongly vary.

1.5. Methods to assess biodegradability on oxidation effluents

The implementation of an oxidation process as a pre-treatment for a conventional or advanced biological wastewater treatment requires the estimation of the biodegradability assessment not only of the raw wastewater

but to the oxidation effluents as well. Among the most common techniques to assess biodegradability is the 5-day biochemical oxygen demand (BOD_5) test or the calculation of the BOD_5/COD ratio. This value provides an approximate index of the proportion of organic substances present in the wastewater that are biodegradable under aerobic conditions. The main limitation of this test is that is consumes a considerable amount of time to obtain the results (Oller et al., 2011). Posada et al (2006) have used the BOD_5/COD ratio to describe the biodegradability increment on PNP CWAO effluent obtaining a 40% of biodegradability increment when compared to the original effluent.

Several studies focus on the toxicity of the recalcitrant compounds instead of the biodegradability of the molecule, those toxicity assays may give results where a chemical compound is toxic to the specific organisms in the bioassay but they are not toxic to bacteria, which promote the degradation process (Rizzo, 2011). In this case the oxygen demand obtained in respirometric assays has recently turned into an excellent control parameter as it represents a direct measure of the correct activity and viability of microorganisms present in aerobic activated sludge. As this test represents a direct assessment of the primary function in a process based on activated sludge, it can be used as an efficient tool for the measure of acute toxicity that could provoke the inlet of different industrial wastewater and its biodegradability on the activated sludge of an urban WWTP (Oller et al., 2011; Rizzo, 2011).

In this thesis, a respirometry method that provides information on the inhibition, biodegradability and toxicity of chemical compounds in an urban WWTP is used (Guisasola et al., 2003) and that has been effectively used to determine the biodegradability of WAO and CWAO effluents from phenolic wastewater (Suárez-Ojeda et al., 2007b).

1.6. Research motivation and thesis overview.

This thesis was developed within the GENOCOV research group (liquid and gas effluents treatment group: nutrients, odor and volatile organic compounds removal) at the Department of Chemical Engineering, Universitat

Autònoma de Barcelona, specifically in the research area of industrial effluents treatment dealing with recalcitrant compounds. GENOCOV is a consolidated group from the Generalitat of Catalonia (reference SGR 2009 815).

This thesis has its background on a previous study (Suárez-Ojeda, 2006) where the objective was to use WAO and CWAO for selected phenolic compounds that are typical in effluents from chemical industry, in order to find the appropriate conditions that guarantee the destruction of the model compound, the preservation of the catalyst, and the increase of the biodegradability of the effluents. So in that case, these processes could be used as a pre-treatment for a subsequent biological degradation in an urban WWTP. It was found that for the different compounds tested, the behavior was not always satisfactory for the same catalyst, leaving as a result that the effectiveness of coupling of a chemical plus biological oxidation process will depend, in general, on the nature of the target pollutant and/or the wastewater composition in the case of a real effluent.

In this thesis, PNP was selected as a model compound with the aim of study the optimum process and operation parameters for its removal from wastewater using several technologies.

In the management of industrial wastewater several things need to be taken in account; for instance, in the chemical manufacturing industry the production is seasonal or by demand, and evidently, different products can be manufactured on the same plant; this means that the effluents composition can be highly variable. Other frequent problem is that the wastewater flow, which is directly related to the volume of production, can also vary according to the demand; this scenario makes difficult the selection of a single process to treat such wastewaters.

Despite all the physical, biological and chemical processes available to treat wastewaters, it is still a difficult task to selected the correct one when recalcitrant compounds are present in industrial wastewater; in this sense, it is necessary to develop efficient and less expensive processes to deal with this problem.

On one hand, chemical oxidation is usually an expensive treatment due to the high cost of the special materials for construction of the reactors and catalysts. On the other hand, biological treatment (even though it could be the most suitable process in terms of economy) requires a long period of time to be implemented for recalcitrant compounds; it could also need larger reactors and a dedicated control system that would increment the running costs of the WWTP and leave it without the flexibility to deal with a transient flow of influent.

So that, in the next chapter the general and specific objectives for this thesis are presented. Afterwards, in chapter 3 the biological treatment of a high-strength PNP wastewater (up to 385 mg L^{-1}) in an aerobic SBR is presented. The start up of the reactor was performed using a non-acclimated biomass coming from an urban WWTP as inoculum and a synthetic wastewater containing PNP with glucose-sucrose as carbon source. The start-up process lasted about 200 days until final desired conditions were reached. In this chapter the enrichment process is described, a kinetic characterization for PNP-degrading population was carried out using respirometry assays that later permitted to perform a kinetic model including the substrate inhibition variable and finally indirect evidence for the PNP oxidation pathway was given. These results have been published in a peer-reviewed journal (Martín-Hernández et al., 2009).

Chapter 4 deals with transient or continuous shock loads of PNP in an aerobic sequencing batch reactor, which is the most common situation in the industry where the production is not continuous. For this case, the bioaugmentation with an enriched microbial population was proposed and several conditions were tested to optimize the process. The effect of the amount of the enriched microbial population added was assessed since it is a critical parameter to be taken in account for the scale-up of the process to a real application. Furthermore, monitoring of the microbial population in the reactor was done by means of fluorescence in-situ hybridization (FISH) coupled with confocal laser scanning microscopy (CLSM) to explain the results. The performance enhancement of a bioaugmented SBR receiving a continuous PNP shock load was also studied in this chapter.

Finally in chapter 5 a different approach is taken in the case of highly concentrated PNP wastewaters (up to 5000 mg L^{-1}) that cannot be degraded in a biological reactor. In this case the concentration of PNP wastewater was 13 times higher than the concentration of PNP wastewaters on chapter 3. In this chapter WAO and CWAO were performed to oxidize the PNP molecule using Pt and Ru-based catalysts and different operation conditions with the aim to increase the biodegradability of the effluents, part of this of the research was done in collaboration with the Institut de Recherches sur la Catalyse et l'Environnement de Lyon (IRCELyon – France) in a 6 months stay, the oxidation pathway was proposed and the results of the performance on different operation conditions, different catalysts and different supports were compared. Respirometric experiments were carried out with the objective of determine the biodegradation parameters to assess if an integrated CWAO and biological treatment would be possible. These results have been published in a peer-reviewed journal (Martín-Hernández et al., 2012). A summary scheme of the topics treated on this thesis is shown on Figure 1.2.

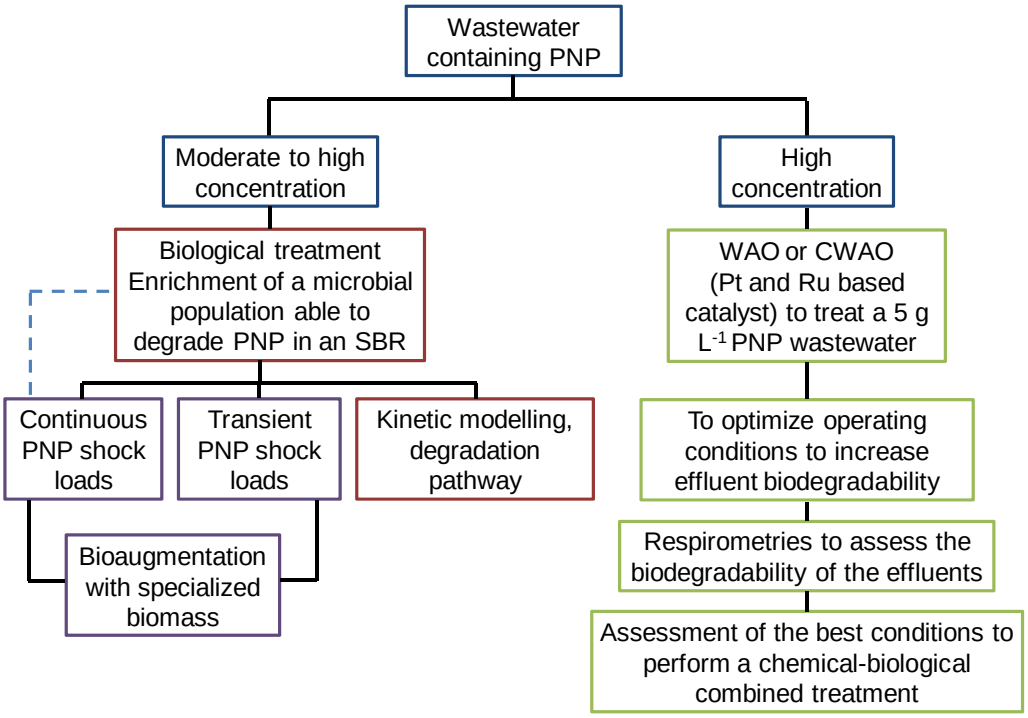


Figure 1.2. Thesis overview. Contents in red are explained in chapter 3, contents in purple are explained in chapter 4 and contents in green are explained in chapter 5.

1.7. References.

91/271/EEC, Council Directive concerning urban waste water treatment. in: *Official Journal of the European Union*. *30-05-1991.*

2010/75/EU, Directive on industrial emissions (integrated pollution prevention and control). *Official Journal of the European Union. 17-12-2010.*

ATSDR. 1992. Toxicological Profile for nitrophenols: 2-Nitrophenol and 4-Nitrophenol. *U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.*

Bhatti, Z.I., Toda, H., Furukawa, K. 2002. p-Nitrophenol degradation by activated sludge attached on nonwovens. *Water Research, 36, 1135-1142.*

Chan, Y., Chong, M., Law, C., Hassell, D. 2009. A review on anaerobic-aerobic treatment of industrial and municipal wastewater. *Chemical Engineering Journal, 155(1-2), 1-18.*

Chong, N., Pai, S., Chen, C. 1997. Bioaugmentation of an activated sludge receiving pH shock loadings. *Bioresource Technology, 59(2-3), 235-240.*

Collado, S., Laca, A., Diaz, M. 2011. Effect of the carboxylic substituent on the reactivity of the aromatic ring during the wet oxidation of phenolic acids. *Chemical Engineering Journal, 166(3), 940-946.*

Crawford, R.L. 1995. The microbiology and treatment of nitroaromatic compounds. *Current Opinion in Biotechnology, 6(3), 329-336.*

Dai, Q., Lei, L., Zhang, X. 2008. Enhanced degradation of organic wastewater containing p-nitrophenol by a novel wet electrocatalytic oxidation process: Parameter optimization and degradation mechanism. *Separation and Purification Technology, 61(2), 123-129.*

E-PRTR. 2012. European Pollutant and Transfer Register for 2010. Available at http://prtr.ec.europa.eu/pgAbout.aspx. *European Environment Agency*. Last time consulted 01/05/2012.

Eichenbaum, G., Johnson, D., Kirkland, P., O'Neil, P., Stellar, S., Bielawne, J., DeWire, R. 2009. Assessment of the genotoxic and carcinogenic risks of p-nitrophenol when is present as an impurity in a drug product.

Regulatory Toxicology and Pharmacology, 55(1), 33-42.

El Fantroussi, S., Agathos, S.N. 2005. Is bioaugmentation a feasible strategy for pollutant removal and site remediation? *Current Opinion in Microbiology, 8(3), 268-275.*

EPA. 2010. List of priority pollutants included on the Appendix A to 40 CFR Pt 423. U.S. Government Printing Office

FAO. 2010. Food and Agriculture Organisation of the United Nations. *Aquastat Online Database.* Available at:

http://www.fao.org/nr/water/aquastat/dbase/AquastatWorldDataEng_20101129.pdf. Last time consulted 01/05/2012.

Guisasola, A., Baeza, J., Carrera, J., Casas, C., Lafuente, J. 2003. An off-line respirometric procedure to determine inhibition and toxicity of biodegradable compounds in biomass from an industrial WWTP. *Water Science and Technology, 48(11-12), 267-275.*

Hu, X., Li, A., Fan, J., Deng, C., Zhang, Q. 2008. Biotreatment of p-nitrophenol and nitrobenzene in mixed wastewater through selective bioaugmentation. *Bioresource Technology, 99(10), 4529-4533.*

Jain, R.K., Dreisbach, J.H., Spain, J.C. 1994. Biodegradation of *p*-nitrophenol via 1,2,4-benzenetriol by an *Arthrobacter* sp. *Applied and Environmental Microbiology*, 60(8), 3030-3032.

Jianlong, W., Xiangchun, Q., Libo, W., Yi, Q., Hegemann, W. 2002. Bioaugmentation as a tool to enhance the removal of refractory compound in coke plant wastewater. *Process Biochemistry, 38(5), 777-781.*

Kadiyala, V., Smets, B., Chandran, K., Spain, J. 1998. High affinity p-nitrophenol oxidation by Bacillus sphaericus JS905. *Fems Microbiology Letters, 166(1), 115-120.*

Karim, K., Gupta, S. 2002. Effects of alternative carbon sources on biological transformation of nitrophenols. *Biodegradation, 13(5), 353-360.*

Kim, K., Ihm, S. 2011. Heterogeneous catalytic wet air oxidation of refractory organic pollutants in industrial wastewaters: A review. *Journal of Hazardous Materials, 186(1), 16-34.*

Klavarioti, M., Mantzavinos, D., Kassinos, D. 2009. Removal of residual pharmaceuticals from aqueous systems by advanced oxidation processes. *Environment International, 35(2), 402-417.*

Kulkarni, M., Chaudhari, A. 2007. Microbial remediation of nitro-aromatic compounds: An overview. *Journal of Environmental Management, 85(2), 496-512.*

Kuscu, O., Sponza, D. 2009. Kinetics of para-nitrophenol and chemical oxygen demand removal from synthetic wastewater in an anaerobic migrating blanket reactor. *Journal of Hazardous Materials, 161(2-3), 787-799.*

Levec, J., Pintar, A. 2007. Catalytic wet-air oxidation processes: A review. *Catalysis Today, 124(3-4), 172-184.*

Li, N., Descorme, C., Besson, M. 2007. Catalytic wet air oxidation of 2-chlorophenol over Ru loaded CexZr1-xO2 solid solutions. *Applied Catalysis B-Environmental, 76(1-2), 92-100.*

Liou, R., Chen, S., Huang, C., Lai, C., Shih, C., Chang, J., Hung, M. 2010. Catalytic wet peroxide oxidation of p-nitrophenol by Fe (III) supported on resin. *Water Science and Technology, 62(8), 1879-1887.*

Loos, R., Gawlik, B., Locoro, G., Rimaviciute, E., Contini, S., Bidoglio, G. 2009. EU-wide survey of polar organic persistent pollutants in European river waters. *Environmental Pollution, 157(2), 561-568.*

Löser, C., Oubelli, M., Hertel, T. 1998. Growth kinetics of the 4-nitrophenol degrading strain Pseudomonas putida PNP1. *Acta Biotechnologica*, 18(1), 29-41.

Mantzavinos, D., Sahibzada, M., Livingston, A., Metcalfe, I.S., Hellgardt, K. 1999. Wastewater treatment: wet air oxidation as a precursor to biological treatment. *Catalysis Today, 53(1), 93-106.*

Martín-Hernández, M., Carrera, J., Pérez, J., Suárez-Ojeda, M.E. 2009. Enrichment of a K-strategist microbial population able to biodegrade p-nitrophenol in a sequencing batch reactor. *Water Research, 43(15), 3871-3883.*

Martín-Hernández, M., Carrera, J., Suárez-Ojeda, M.E., Besson, M., Descorme, C. 2012. Catalytic wet air oxidation of a high strength p-nitrophenol wastewater over Ru and Pt catalysts: Influence of the reaction conditions on biodegradability enhancement. *Applied Catalysis B: Environmental, 123-124, 141-150.*

Mishra, K., Gogate, P. 2011. Intensification of sonophotocatalytic degradation of p-nitrophenol at pilot scale capacity. *Ultrasonics Sonochemistry, 18(3), 739-744.*

Mrozik, A., Piotrowska-Seget, Z. 2010. Bioaugmentation as a strategy for cleaning up of soils contaminated with aromatic compounds. *Microbiological Research, 165(5), 363-375.*

NITE. 2007. The 2005 PRTR Data report. Total releases and transfers. *National Institute of Technology and Evaluation. Japan.*

NLM. 1992. 4-Nitrophenol profile. *U.S. National Library of Medicine. Available at:* http://toxmap.nlm.nih.gov/toxmap/main/chemPage.jsp?chem=4-Nitrophenol. Last time consulted: 27/04/2011

OECD. 2008. The 2004 Organisation for Economic Co-operation and Development (OECD) List of High Production Volume Chemicals. http://www.oecd.org/dataoecd/55/38/33883530.pdf. Last time consulted: 26/04/2012.

Oller, I., Malato, S., Sánchez-Pérez, J.A. 2011. Combination of Advanced Oxidation Processes and biological treatments for wastewater decontamination-A review. *Science of The Total Environment, 409(20), 4141–4166.*

Pintar, A., Levec, J. 1994. Catalytic-Oxidation of Aqueous p-Chlorophenol and p-Nitrophenol Solutions. *Chemical Engineering Science, 49(24A), 4391-4407.*

Posada, D., Betancourt, P., Liendo, F., Brito, J. 2006. Catalytic wet air oxidation of Aqueous solutions of substituted phenols. *Catalysis Letters, 106(1-2), 81-88.*

Protection of the Environment. 2010. Title 40. Code of Federal Regulation Pt. 414. *U.S. Government Printing Office.*

Qiu, X., Bai, W., Zhong, Q., Li, M., He, F., Li, B. 2006. Isolation and characterization of a bacterial strain of the genus Ochrobactrum with methyl parathion mineralizing activity. *Journal of Applied Microbiology, 101(5), 986-994.*

Quan, X., Shi, H., Liu, H., Wang, J., Qian, Y. 2004. Removal of 2,4-dichlorophenol in a conventional activated sludge system through bioaugmentation. *Process Biochemistry, 39(11), 1701-1707.*

Ray, P., Oubelli, M., Löser, C. 1999. Aerobic 4-nitrophenol degradation by microorganisms fixed in a continuously working aerated solid-bed reactor. *Applied Microbiology and Biotechnology, 51(2), 284-290.*

Razo-Flores, E.A., Donlon, B., Lettinga, G., Field, J.A. 1997. Biotransformation and biodegradation of N-substituted aromatics in methanogenic granular sludge. *FEMS Microbiology Review 20(3-4), 525 - 538.*

Rezouga, F., Hamdi, M., Sperandio, M. 2009. Variability of kinetic parameters due to biomass acclimation: Case of para-nitrophenol biodegradation. *Bioresource Technology, 100(21), 5021-5029.*

Rizzo, L. 2011. Bioassays as a tool for evaluating advanced oxidation processes in water and wastewater treatment. *Water Research, 45, 4311-4340.*

Sahoo, N., Pakshirajan, K., Ghosh, P. 2011. Biodegradation of p-nitrophenol using Arthrobacter chlorophenolicus A6 in a novel upflow packed bed reactor. *Journal of Hazardous Materials, 190(1-3), 729-737.*

Salehi, Z., Yoshikawa, H., Mineta, R., Kawase, Y. 2011. Aerobic biodegradation of p-nitrophenol by acclimated waste activated sludge in a slurry bubble column. *Process Biochemistry, 46(1), 284-289.*

Santos, A., Yustos, P., Rodriguez, S., Garcia-Ochoa, F. 2006. Wet oxidation of phenol, cresols and nitrophenols catalyzed by activated carbon in acid and basic media. *Applied Catalysis B-Environmental, 65(3-4), 269-281.*

Sigma-Aldrich. 2012. Material safety data sheet. p-nitrophenol. According to Regulation 1907/2006/EC.

Singer, A., van der Gast, C., Thompson, I. 2005. Perspectives and vision for strain selection in bioaugmentation. *Trends in Biotechnology, 23(2), 74-77.*

Spain, J., Gibson, D. 1991. Pathway for biodegradation of para-nitrophenol in a Moraxella SP. *Applied and Environmental Microbiology, 57(3), 812-819.*

Sponza, D., Kuscu, O. 2005. p-nitrophenol removal in a sequential anaerobic migrating blanket reactor (AMBR)/aerobic completely stirred tank reactor (CSTR) system. *Process Biochemistry, 40(5), 1679-1691.*

Stüber, F., Font, J., Eftaxias, A., Paradowska, M., Suárez, M., Fortuny, A., Bengoa, C., Fabregat, A. 2005. Chemical wet oxidation for the abatement of refractory non-biodegradable organic wastewater pollutants. *Process Safety and Environmental Protection, 83(B4), 371-380.*

Suárez-Ojeda, M.E., Stuber, F., Fortuny, A., Fabregat, A., Carrera, J., Font, J. 2005. Catalytic wet air oxidation of substituted phenols using activated carbon as catalyst. *Applied Catalysis B-Environmental, 58(1-2), 105-114.*

Suárez-Ojeda, M.E. 2006. Catalytic wet air oxidation coupled with an aerobic biological treatment to deal with an industrial wastewater, PhD Thesis. *Universitat Rovira i Virgili.*

Suárez-Ojeda, M.E., Guisasola, A., Baeza, J.A., Fabregat, A., Stuber, F., Fortuny, A., Font, J., Carrera, J. 2007a. Integrated catalytic wet air oxidation and aerobic biological treatment in a municipal WWTP of a high-strength o-cresol wastewater. *Chemosphere, 66(11), 2096-2105.*

Suárez-Ojeda, M.E., Kim, J., Carrera, J., Metcalfe, I., Font, J. 2007b. Catalytic and non-catalytic wet air oxidation of sodium dodecylbenzene sulfonate: Kinetics and biodegradability enhancement. *Journal of Hazardous Materials, 144(3), 655-662.*

Suárez-Ojeda, M.E., Montón, H., Roldán, M., Martín-Hernández, M., Pérez, J., Carrera, J. 2011. Characterization of a p-nitrophenol-degrading mixed culture with an improved methodology of fluorescence in situ hybridization and confocal laser scanning microscopy. *Journal of Chemical Technology and Biotechnology*, 86(11), 1405-1412

Tomei, M., Annesini, M., Luberti, R., Cento, G., Senia, A. 2003. Kinetics of 4-nitrophenol biodegradation in a sequencing batch reactor. *Water Research, 37(16), 3803-3814.*

TRI. 2012. Chemical releases and transfers for 2010. Toxic Release Inventory. *Environmental Protection Agency. U.S.*

UNESCO. 2012. World Water Assessment Programme (WWAP). The United Nations World Water Development Report 4: Managing Water under Uncertainty and Risk. Paris.

Vogel, T. 1996. Bioaugmentation as a soil bioremediation approach. *Current Opinion in Biotechnology, 7(3), 311-316.*

Wang, J., Zhao, G., Wu, L. 2005. Slurry-phase biological treatment of nitrophenol using bioaugmentation technique. *Biomedical and Environmental Sciences, 18(2), 77-81.*

Xing, X., Inoue, T., Tanji, Y., Unno, H. 1999. Enhanced microbial adaptation to p-nitrophenol using activated sludge retained in porous carrier particles and, simultaneous removal of nitrite released from degradation of p-nitrophenol. *Journal of Bioscience and Bioengineering, 87(3), 372-377.*

Yi, S., Zhuang, W.-Q., Wu, B., Tiong-Lee Tay, S., Tay, J.-H. 2006. Biodegradation of p-nitrophenol by aerobic granules in a sequencing batch reactor. *Environmental Science & Technology, 40, 2396-2401.*

Zhang, H., Fei, C., Zhang, D., Tang, F. 2007. Degradation of 4-nitrophenol in aqueous medium by electro-Fenton method. *Journal of Hazardous Materials, 145, 227-232.*

CHAPTER 2

Objectives

The main objective of this thesis was to make a comparative study of several treatment strategies for the remediation of a high-strength p-nitrophenol (PNP) wastewater. Therefore, different approaches were considered with the aim of proposing a best available technique for the treatment of recalcitrant compounds at industrial level. This includes different scenarios according to the transient or continuous PNP occurrence in the wastewater and also for the case of highly concentrated wastewater.

Following this idea, the specific objectives for this thesis were:

- To treat biologically a high-strength PNP wastewater in an aerobic sequencing batch reactor (SBR).
- To implement a feeding strategy allowing a stable operation of the SBR and for developing of a K-strategist PNP-degrading sludge suitable for bioaugmentation.
- To evaluate the benefits of using bioaugmentation for PNP removal following transient or continuous PNP shock loads in a non-acclimated SBR.
- To study the persistence of the specialized PNP-degrading bacteria inside the bioaugmented SBR and its ability to degrade a transient PNP shock load after several sludge retention times.
- To treat a highly-concentrated PNP wastewater using CWAO and supported Ru and Pt catalysts to increase the biodegradability of the influent before a subsequent biological treatment.
- To establish the most suitable conditions for a combined CWAO and biological treatment of PNP contaminated wastewater.

CHAPTER 3

Enrichment of a K-strategist microbial population able to biodegrade *p*-nitrophenol in a sequencing batch reactor

The content of this chapter has been published as: **Martín-Hernández, M.**, Carrera, J., Pérez, J. Suárez-Ojeda, M.E., 2009. Enrichment of a K-strategist microbial population able to biodegrade *p*-nitrophenol in a sequencing batch reactor. Water Research, 43, 3871-3883

The calibration and validation of the mathematical model for the kinetic characterization of the PNP-degrading activated sludge was developed by Dr. Julio Pérez.

Abstract

The biological treatment of a high-strength *p*-nitrophenol (PNP) wastewater in an aerobic Sequencing Batch Reactor (SBR) has been studied. A specific operational strategy was applied with the main aim of developing a K-strategist PNP-degrading activated sludge. The enrichment of a K-strategist microbial population was performed using a non-acclimated biomass coming from a municipal WWTP as inoculum, and following a feeding strategy in which the PNP-degrading biomass was under endogenous conditions during more than 50% of the aerobic reaction phase. Hundred per cent of PNP removal was achieved in the whole operating period with a maximum specific PNP loading rate of 0.26 g PNP g^{-1} VSS d^{-1}. A kinetic characterization of the obtained PNP-degrading population was carried out using respirometry assays in specifically designed batch tests. With the experimental data obtained a kinetic model including substrate inhibition has been used to describe the time-course of the PNP concentration and specific oxygen uptake rate (SOUR), simultaneously. The kinetic parameters obtained through optimization, validated with an additional respirometric test, were k_{max}= 1.02

mg PNP mg^{-1} COD d^{-1}, K_s= 1.6 mg PNP L^{-1} and K_i = 54 mg PNP L^{-1}. The values obtained for the K_s and k_{max} are lower than those reported in the literature for mixed populations, meaning that the biomass is a K-strategist type, and therefore demonstrating the success of the operational strategy imposed to obtain such a K-strategist population. Moreover, our measured K_i value is higher than those reported by most of the bibliographic references; therefore the acclimated activated sludge used in this work was evidently more adapted to PNP inhibition than the other reported cultures.

3.1. Introduction

As revealed by its inclusion in the catalogue of High Volume Production Chemicals by the Organization for Economic Cooperation and Development (OECD, 2008), one of the most widely used nitrophenolic compounds is *p*-nitrophenol (PNP). PNP has important applications in agriculture, in the dyes and pigment industry and in polymers and pharmaceutical products. PNP is also used as a fungicide for leather, in the production of methyl parathion and in organic synthesis (Bhatti et al., 2002). Despite its wide uses, PNP is highly toxic both for the environment and humans; repeated exposure may cause injury to blood cells, damage to the central nervous system and mutagenic effects; whereas long-term exposure could cause extensive damage to kidney and liver (ATSDR, 1992). Furthermore, due to its high solubility in water, PNP is a potential environmental contaminant of water reservoirs and soils. It is classified as a priority pollutant by the United States Environmental Protection Agency (EPA, 2009).

Since a technique for its efficient removal is needed, great effort has been invested in this area of research. Chemical treatments such as wet air oxidation require high temperatures and oxygen pressures (Bhargava et al., 2006; Dai et al., 2008), resulting in high running costs. As an alternative, biological treatments have been proposed. Anaerobic treatment of PNP results in aromatic amines as intermediates; these require further aerobic treatment (Razo-Flores et al., 1997). Aerobic treatment could lead to complete mineralization of PNP and it has been tested for wastewater treatment (Ray et

al., 1999) and bioremediation of contaminated soils (Labana et al., 2005a). Several studies have focused on the isolation and characterization of aerobic PNP-degrading microbial strains and their pathways (Bhushan et al., 2000; Jain et al., 1994; Kadiyala et al., 1998; Labana et al., 2005b; Liu et al., 2007; Loser et al., 1998; Spain and Gibson, 1991; Wan et al., 2007). Nevertheless, from a practical point of view, a full-scale application with isolated strains for aerobic PNP biodegradation would be difficult. Consequently, some studies aimed to obtain PNP-degrading populations starting from a conventional activated sludge and, using controlled enrichment processes and different reactor configuration, such as biofilm, activated sludge and granular biomass (Bhatti et al., 2002; Tomei et al., 2003; Tomei and Annesini, 2005; Xing et al., 1999; Yi et al., 2006). These contributions were focused on the continuous treatment of wastewaters containing considerable PNP concentrations (100-700 mg PNP L^{-1}). However, PNP might only intermittently appear in the influent of some biological wastewater treatments or in contaminated soils. In both situations, bioaugmentation or the introduction of an external PNP-degrading population in a contaminated environment could be a good approach to provide remediation at a faster rate than would otherwise occur by means of the indigenous microorganisms (Gentry et al., 2004; Hu et al., 2008; Singer et al., 2005).

It is proposed that bioaugmentation would be more effective if the PNP-degrading microbial population has high affinity for PNP (Juteau et al., 1999; Labana et al., 2005b). In other words, bioaugmentation would be more efficient if the PNP-degrading population is K-strategist type. In the ecological concept of r- and K-selection, the microorganisms are classified according to their competitive abilities to survive. An r-strategist microorganism shows quick growth on easily available substrates and a K-strategist microorganism grows slowly but, using the limited resources more efficiently, is capable of surviving long periods of starvation (Andrews and Harris, 1986). The ecological concept of r- and K-selection can be applied at a finer scale to the kinetics of a microbial population. For example, in a Monod's kinetic model, a K-strategist population will have lower substrate removal rate and higher affinity for substrate (or lower half-saturation coefficient) than an r-strategist

population (Blackburne et al., 2007; Dytczak et al., 2008). On the other hand, the PNP-degrading microorganisms suffer substrate inhibition by PNP (Tomei et al., 2003; Yi et al., 2006). Thus, bioaugmentation would be more efficient if the PNP-degrading population was acclimated to high PNP concentrations. The biological concept of substrate inhibition can be also applied at a finer scale to the kinetics of a microbial population. For example, in a Haldane's kinetic model (Andrews, 1968), a microbial population acclimated to the inhibitory substrate will have a higher substrate inhibition constant than a non-acclimated population (Antileo et al., 2002; Jubany et al., 2005).

Therefore, the objectives of this work were as follows.

- To treat a high-strength PNP wastewater biologically in an aerobic Sequencing Batch Reactor (SBR).
- To develop a K-strategist PNP-degrading activated sludge adequate for bioaugmentation treatments.

The starting points were a non-acclimated activated sludge and a feeding strategy based on maintaining long periods under endogenous conditions during the SBR cycle. Finally, a kinetic study has been performed in order to confirm the attainment of a K-strategist population.

3.2. Materials and methods

3.2.1. Chemicals and analytical methods

PNP in granular form (purity 98%) was used. It was supplied by Sigma-Aldrich (Spain), as were all other chemicals used in this work. The highest purities available were employed.

Total and volatile suspended solids (TSS and VSS) were determined using the procedure stated in Standard Methods (APHA, 1998). Total organic carbon (TOC) was measured with an OI Analytical TOC Analyzer (Model 1020A) equipped with a non-dispersive infrared (NDIR) detector and a furnace maintained at 680ºC.

PNP and metabolic intermediates concentrations were determined by

HPLC, (UltiMate 3000, Dionex Corporation) using an Agilent Zorbax SB-C18 (4.6 x 100 mm, 3.5 μm) column and a UV detector at 254 nm (290 nm for 1,2,4-benzenetriol), the flow rate was set at 1.875 mL min^{-1} and the column temperature was set at 30ºC. The mobile phases were ultrapure water containing H_2SO_4 at pH 1.41 and HPLC grade methanol following a gradient elution. The gradient started from 100 % of acidified water and progressively changed to 50:50 v/v of acid water:methanol in 18 minutes, then it remained isocratic until minute 20. The injection volume was 20 μL and the maximum pressure in the column was approximately 290 bar. Samples were filtered with 0.45 μm syringe filter driven unit from Milipore® provided with a high-density polyethylene housing and membrane of hydrophilic Durapore® (PVDF). Calibration was performed by means of external standards of PNP and of other compounds reported as intermediates such as hydroquinone (HQ), 4-nitrocatechol (4-NC) and 1,2,4-benzenetriol (1,2,4-BT) (Jain et al., 1994; Spain and Gibson, 1991).

Nitrite and nitrate concentrations were determined by ionic chromatography (IC) using an ICS-2000 Integrated Reagent-Free IC system (Dionex Corporation), which performs ion analyses using suppressed conductivity detection. An IonPac AS18 analytical column plus an AG18 guard column and an anion self-regenerating suppressor (ASRS ULTRA II) were used. The eluent was potassium hydroxide generated from an EGC KOH cartridge following a gradient elution. The gradient started from 18 mM of KOH and progressively changed to 50 mM in 10 min. After that it decreased to 18 mM per minute and then remained isocratic until minute 15. The injection volume was 25 μL. Column temperature was set at 30ºC and samples were filtered with a 0.20 μm syringe filter driven unit from Millipore® provided with a high-density polyethylene housing and membrane of hydrophilic Durapore® (PVDF).

3.2.2. Experimental setup

A process diagram of the SBR is shown in Figure 3.1. The SBR was a 20 L stainless steel tank (SBR-1), equipped with a mechanical stirrer (M-1), a

compressed air inlet (C-1) and a heating device. The reactor had two valves for liquid sampling and one emergency valve to prevent reactor flooding. Feeding to the reactor was made with a membrane pump (P-1), effluent draw was performed using an electrical valve (V-2) and sludge purge with a manual valve (V-1). Temperature in the reactor was maintained using a thermistor and a temperature controller (TC-1). The reactor was also equipped with dissolved oxygen (DO) (DOI-1), temperature (TI-1) and pH (pHI-1) sensors for process monitoring. The SBR was monitored and controlled with software developed in Labview 8.0 (National Instruments). The communication between the PC and the SBR hardware was performed using a compact field point (cFP) system (National Instruments, Spain), which had a cFP-1804 as the Ethernet interface and several input/output modules (cFP-AI-110 and cFP-RLY-421).

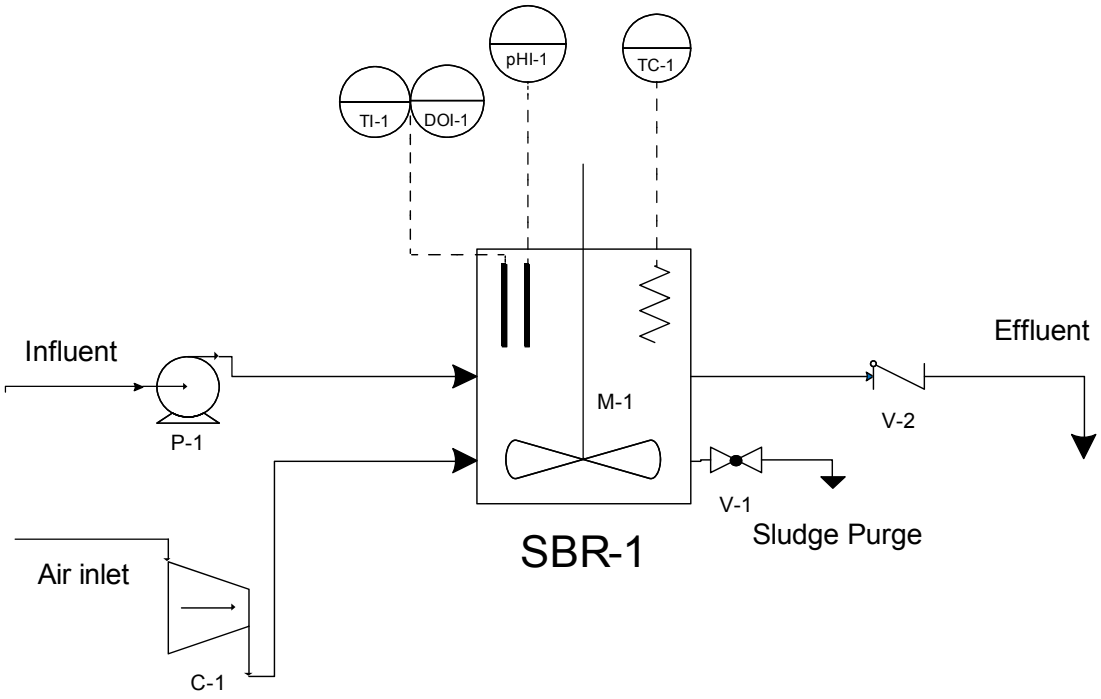


Figure 3.1. SBR Diagram. SBR-1: 20 L stainless steel tank. C-1: Compressed air. M-1: mechanical stirrer. P-1: membrane pump for feeding. PHI-1: pH sensor and indicator. ODI-1: dissolved oxygen (DO) sensor and indicator. TC-1: temperature controller. TI-1: temperature sensor and indicator. V-1: manual valve for sludge purge. V-2: electrical valve for effluent drawing.

The feeding tank was insulated and refrigerated to keep the feed at 10 ºC, the tank was also provided with a mechanical stirrer to guarantee

homogeneity of the feed mixture.

The SBR was inoculated with activated sludge from a full-scale municipal wastewater treatment plant (WWTP) located in Manresa (Barcelona, Spain). The sludge was adapted to pilot plant conditions during a 30-day period using a synthetic wastewater without PNP. Glucose was used as the main organic component of this synthetic influent. Other components and micronutrients were (concentrations are expressed in mg L^{-1}): KH_2PO_4 (41), $CaCl_2$ (88), $CO(NH_2)_2$ (12), NH_4Cl (106), yeast extract (2), NaCl (176), $MgCl_2 \cdot 6H_2O$ (198), $FeSO_4$ (4), $MnSO_4 \cdot 4H_2O$ (3), $ZnSO_4 \cdot 7H_2O$ (4), $CuSO_4 \cdot 5H_2O$ (2) and H_3BO_3 (0.02). After this first period, the percentage of PNP in the influent of the SBR was progressively increased following the sequence described in Table 3.1. The total TOC influent concentration was always maintained between 300 and 400 mg L^{-1} whereas the PNP influent concentration was increased to almost 400 mg L^{-1} in the final period.

Table 3.1. Carbon substrate concentrations and PNP:glucose ratios in the SBR influent.

PNP:glucose (ratio in terms of TOC)	0:100	7.5:92.5	25:75	40:60	60:40
[PNP] (mg L^{-1})	0	38 ± 6	133 ± 19	231 ± 9	367 ± 22
[TOC] (mg L^{-1})	294 ± 41	293 ± 39	324 ± 16	345 ± 18	368 ± 23

Two different operating schemes were used throughout the whole experimental period. During the first 181-day period (0-181 days), the operation was established in cycles of 6 h divided into an aerobic reaction phase of 4.8 h (the fill phase was completed in the first 15 min of this phase), a setting phase of 60 min, a draw phase of 7 min and an idle phase of 5 min. The exchange volume in each cycle was 50% of the total volume. Therefore, the hydraulic retention time (HRT) in this period was 0.5 days.

The operation in the last 169-day period (181-350 days) was established in cycles of 24 h divided into an aerobic reaction phase of 22.8 h (the filling phase was performed in 8 sub-phases of 1.9 min each, during the first 20 h of

the aerobic reaction phase), a settling phase of 60 min, a draw phase of 7 min and an idle phase of 5 min. The exchange volume in each cycle was 50% of the total volume; therefore, the HRT in this period was 2.0 days. Purge was calculated for maintaining the sludge retention time (SRT) at 20 days during the whole experimental period. The pH was adjusted to 7.5 by adding a controlled quantity of sodium bicarbonate to the feed, whereas temperature was maintained with an on-off controller at 19-20ºC. DO concentration was in the range [6.5 -7.5] mg O_2 L^{-1} although a closed control loop for DO was not implemented.

3.2.3. Respirometry

Respirometric tests were made for the evaluation of kinetic parameters and to investigate the PNP degradation pathway. The respirometer used in this work was a LFS (liquid-flow-static) type, where DO is measured in the liquid-phase which is static and continuously aerated (Spanjers et al., 1998). The vessel (1L) was magnetically stirred and the air flowed through a pressure manoreductor and a gas flowmeter to ensure a constant airflow. The respirometer was provided with pH and DO sensors, a microburette and a flowmeter that were connected to a PC with Windows XP environment; a program developed in Visual Basic (v6.0) was used to control the system. The respirometry measurements were carried out at a constant temperature of 26.0 ± 0.5 ºC and the pH was maintained at 7.5 ± 0.5. Test conditions were adapted from another study (Suárez-Ojeda et al., 2007). The oxygen uptake rate (OUR) profile was obtained by performing a DO balance in the liquid phase of the respirogram.

For each respirometric test, biomass was taken from the SBR during the last part of the aerobic phase once the PNP was completely depleted. In the kinetic tests, an initial pulse of PNP (60 or 125 mg L^{-1}) as the sole organic carbon source was added and its degradation was followed by monitoring the DO and the PNP concentration from samples taken during the respirometry. In the tests to investigate the degradation pathway, the same procedure was followed but using a pulse of the metabolic intermediate (HQ or 4-NC) as the

sole organic carbon source. One milliliter of samples for HPLC analysis were taken every ca. 10-20 min.

3.2.4. Model development

An ASM-based model (Henze et al., 2000) was used for describing the biological removal of PNP by heterotrophic bacteria. This process was mathematically described according to a substrate inhibition model, Haldane kinetics (Andrews, 1968), as previously reported by other researchers (Tomei et al., 2003; Yi et al., 2006). The model considers two variables: oxygen concentration ([*DO*] in mg O_2 L^{-1}) and PNP concentration ([*PNP*] in mg PNP L^{-1}), both measured in the batch respirometric tests. The differential equations describing the mass balances for PNP and DO consumption by heterotrophic bacteria are:

$$\frac{d[PNP]}{dt} = -k_{\max} \cdot \frac{[PNP]}{K_s + [PNP] + \frac{[PNP]^2}{K_i}} \cdot X \qquad \text{Equation 3.1}$$

$$\frac{d[DO]}{dt} = -\left(1 - Y_{X/PNP}\right) \cdot k_{\max} \cdot \frac{[PNP]}{K_s + [PNP] + \frac{[PNP]^2}{K_i}} \cdot X \qquad \text{Equation 3.2}$$

$$k_{\max} = \frac{\mu_{\max}}{Y_{X/PNP}} \qquad \text{Equation 3.3}$$

$$SOUR = \frac{-\frac{d[DO]}{dt}}{X} \cdot 1.42 \qquad \text{Equation 3.4}$$

where k_{max} is the maximum specific PNP removal rate (in mg PNP mg^{-1} COD min^{-1}), K_s is the half-saturation coefficient for PNP (in mg PNP L^{-1}) and K_i is the substrate inhibition constant for PNP (in mg PNP L^{-1}). *SOUR* is the specific OUR (in mg O_2 g^{-1} VSS min^{-1}), 1.42 in Eq. (3.4) represents the ratio between COD and VSS (considering the biomass as $C_5H_7NO_2$), μ_{max} is the maximum specific growth rate for heterotrophic bacteria (in min^{-1}), $Y_{X/PNP}$ is the yield coefficient for PNP biodegradation (in mg COD mg^{-1} PNP) and X is the total biomass concentration (in mg COD L^{-1}), The total biomass

concentration (*X*) was considered constant during the respirometry due to the slow growth rate of the biomass.

3.2.5. Mathematical methods

All calculations were implemented in MATLAB® v7 R14 SP2 (The Mathworks, Inc, 2005). Eq. (3.1) was solved using a variable order solver based on the numerical differentiation formulas (NDFs) through the MATLAB® function *ode15s* (MATLAB, 1999)

In the parameter estimation section a weighted least squares objective function was used:

$$G\left(\vec{\theta},\vec{w}\right)=\sum_{i=1}^{n} w_i \cdot norm_i\left(\vec{y}^{\exp}-\vec{y}^{\mathrm{model}}(\vec{\theta})\right) \qquad \text{Equation 3.5}$$

where the array $\vec{\theta}=(\theta_1,\theta_2,\theta_3)$ is the vector of model parameters, with $\theta_1=k_{\max}$; $\theta_2=K_s$; $\theta_3=K_i$; w_i are the weighting coefficients used for each one of the data sets included in the objective function, and grouped in vector $\vec{w}$; $norm_i$ is the Euclidean norm of a vector, defined as $norm\left(\vec{X}\right)=\sqrt{\sum_{i=1}^{n} x_i^2}$ for each data set included in the objective function; $\vec{y}^{\exp}$is the vector of the experimental data of each data set and $\vec{y}^{\mathrm{model}}(\vec{\theta})$ is the vector containing the modelling results evaluated for a specific set of parameters $\vec{\theta}$.

To minimize Eq. (3.5), a heuristic multidimensional unconstrained nonlinear optimization method, the so-called Nelder-Mead method, was used, also available in MATLAB® through the function *fminsearch* (MATLAB, 1999). To interpolate the modelling results for PNP concentration and SOUR at the different experimental sampling times, a cubic spline polynomial interpolation algorithm was used (MATLAB, 1999), implemented through the MATLAB® function *interp1*.

For identifiability studies in different parameter estimation problems, the so-called Fisher Information Matrix (FIM) has been successfully used by several researchers. (Dochain and Vanrolleghem, 2001; Jubany et al., 2005;

Marsili-Libelli et al., 2003; Petersen, 2000). The inverse of this matrix yields the parameter covariance matrix $C=F^{-1}$, being the main application linked to the fact that maximizing the FIM implies minimizing the estimation error (Marsili-Libelli and Giusti, 2008). The FIM approach was used in this work to design a specific procedure to optimize the weighting coefficients (w_i) in Eq. (3.5) through the standard error minimization of the parameter K_s (i.e. θ_2), therefore through minimization of the following objective function:

$$J(\vec{w}) = \sigma(\theta_2) = s\sqrt{C_{22}} \qquad \text{Equation 3.6}$$

where $\sigma(\theta_2)$ is the standard error of K_s (i.e. θ_2); s is the residual mean, defined as $s=\sqrt{G\left(\hat{\vec{\theta}},\vec{w}\right)/N-p}$, being $G\left(\hat{\vec{\theta}},\vec{w}\right)$ the objective function in Eq. (3.5) evaluated at the optimum (i.e. $\hat{\vec{\theta}}$) for a certain set of weighting coefficients, $\vec{w}$; N is the number of data points and p the number of parameters (with p=3 because the dimension of $\vec{\theta}$ is three); C_{22} is the diagonal element of the covariance matrix (C) corresponding to K_s (i.e. θ_2). K_s was selected to appear in the objective function (Eq. (3.6)) because it is a key parameter in identifying the kinetics of a microbial population as either r- or K-strategist.

On the other hand, in the multivariate nonlinear parameter estimation problems, the uncertainty analysis is used to estimate the confidence regions. For this purpose the methodology to find the exact confidence regions of the parameters was by way of the following equation (Beale, 1960; Dochain and Vanrolleghem, 2001; Draper and Smith, 1998; Marsili-Libelli and Giusti, 2008):

$$G\left(\vec{\theta},\hat{\vec{w}}\right) = G\left(\hat{\vec{\theta}},\hat{\vec{w}}\right)\cdot\left(1+\frac{p}{N-p}\cdot F(\alpha,p,N-p)\right) \qquad \text{Equation 3.7}$$

where $G\left(\hat{\vec{\theta}},\hat{\vec{w}}\right)$ is the objective function evaluated at the optimum (i.e. $\hat{\vec{\theta}}$ using as weighting coefficients the ones obtained through optimization with Eq. (3.6), i.e., $\hat{\vec{w}}$), p the number of parameters (p=3), N is the total number of data points and F is the value of the F-distribution at a given confidence level α.

Eq. (3.7) was used by simplifying the multidimensional confidence region

to a one-dimensional interval, i.e. the confidence interval for a specific parameter.

3.3. Results and discussion

3.3.1. Enrichment of a K-strategist PNP-degrading activated sludge

The enrichment of a K-strategist PNP-degrading activated sludge was performed using a conventional activated sludge (i.e. non-acclimated biomass) from a municipal WWTP as inoculum, and treating a high-strength PNP wastewater. A key point for developing a K-strategist microbial population in an SBR is the feeding strategy used. Several researchers demonstrated that the development of r- or K-strategist microbial populations can be achieved by controlling the operational strategies in bioreactors (Di Mattia et al., 2002; Dytczak et al., 2008; Ginige et al., 2007; Jih et al., 2008). Considering the r- and K-selection theory (Andrews and Harris, 1986), a SBR operation in which substrate (i.e. PNP) is repeatedly consumed until depletion and then followed by long starvation periods would enhance the development of a K-strategist population (Blackburne et al., 2007). Based on this approach, the SBR feeding strategy was designed to apply cycles with long endogenous periods (i.e. without PNP availability), during the whole operational period.

The operational conditions in the SBR during 350 days of operation are shown in Table 3.2. TOC and PNP concentrations in the influent and effluent of the SBR during the whole operation period are shown in Figure 3.2. The total TOC concentration corresponded to the sum of the PNP and the glucose used as co-substrate. The selection of glucose as co-substrate for PNP biodegradation was based on previous studies (Bhatti et al., 2002; Yi et al., 2006). The first stage of the SBR operation corresponded to a 31-day period of acclimation to the synthetic PNP-free wastewater (Figure 3.2). From day 31 onwards, a progressive increase of PNP concentration in the influent was applied. As expected, a slight accumulation of PNP occurred during the first days (see Figure 3.2b) before the biomass started to consume PNP. After this, 100% PNP removal was attained with the exception of days 150 and

160, when two other PNP accumulations were produced. We explain these below (see Figure 3.2b).

Since the TOC values in the effluent were not negligible, the presence of intermediates of the PNP degradation pathway was explored by HPLC. The calibrated compounds were 4-NC, HQ and 1,2,4-BT, as described previously in Section 3.2. The results obtained from the HPLC analysis systematically showed that none of these intermediates were detected in the effluent. Other references reported low values of TOC for bioremediation systems with 100% of PNP removal efficiency (Tomei et al., 2003; Yi et al., 2006) and they related this organic matter to biomass lysis products.

Table 3.2. SBR operational conditions

% of TOC as PNP	Period (days)	$[VSS]_{SBR}$ (mg L^{-1})	$[TSS]_{eff}$ (mg L^{-1})	HRT (days)	Specific PNP loading rate (g PNP g^{-1} VSS d^{-1})	Specific TOC loading rate (g TOC g^{-1} VSS d^{-1})
0	0-31	4600	75	0.5	0	0.13
7.5	31-86	3500	155	0.5	0.02	0.17
25	86-110	2500	210	0.5	0.11	0.26
40	110-181	1800	92	0.5	0.26	0.38
60	181-350	1300	60	2.0	0.14	0.14

Nitrite and nitrate concentration measurements (data not shown) were carried out at the end of the reaction phase. The results showed that the formation of nitrogenous compounds was stoichiometrically related to PNP disappearance.

The feeding strategy followed from day 0 to 181 consisted of performing the filling phase during the first 15 min of the aerobic reaction phase (4.8 h). This resulted in an operational strategy, which imposed endogenous conditions during 50% of the total reaction phase time.

On days 110-181, the influent contained 40% of the total TOC as PNP. At this stage, a maximum PNP loading rate was achieved (see Table 3.2) but

this high loading rate resulted in two important reactor failures with significant PNP accumulations (days 150 and 160 in Figure 3.2b). These failures were caused by an excessive loading rate that resulted in a PNP accumulation in the SBR and consequently, a substrate inhibition of the PNP-degrading activated sludge was observed. To overcome this problem, we needed to completely stop the SBR feeding until the accumulated PNP was completely removed (this required an aerobic reaction phase of 3-4 days). Similar failures caused by PNP accumulation and the latter effect of substrate inhibition has also been reported for continuous PNP-removing systems (Bhatti et al., 2002; Ray et al., 1999). Therefore, a significant reduction of the applied loading rate was imposed by increasing the HRT up to 2 days from day 181 onwards (see Table 3.2 for detailed figures), after the failures on days 150 and 160.

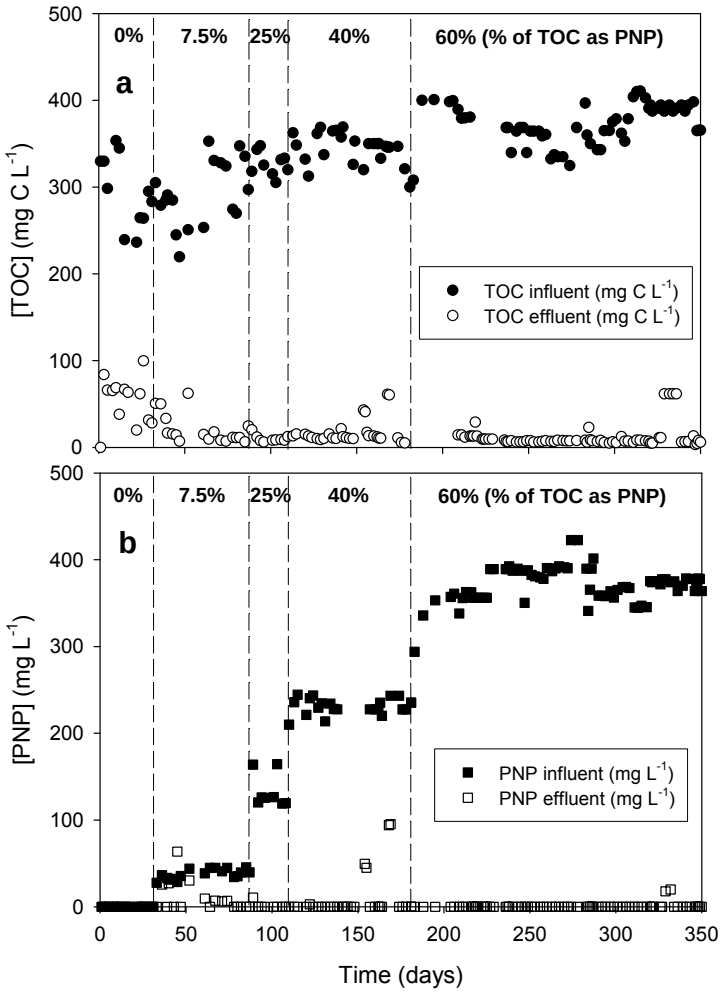


Figure 3.2. TOC and PNP concentrations in the influent and the effluent for the 350 days of the SBR operation.

In addition, a new feeding strategy was implemented on day 181 distributing the PNP fed in eight equal additions along the first 20 h of aerobic reaction phase, this results in 8 sub-phases periods of 20/8=2.5 h each (as can be seen in Figure 3.3). Each one of these additions, every 2.5 h, was performed as rapidly as possible, using a dosing pump in only 1.9 min. The objective of this change was to reduce the PNP concentration at the beginning of the SBR cycle to minimize the substrate inhibition by PNP.

From day 181 onwards, the highest PNP concentration in the influent was achieved (367 mg PNP L^{-1}, see Table 3.1), as a consequence of the new loading rate (0.14 g PNP g^{-1} VSS d^{-1}) and of the new feeding strategy. In the same period, the influent TOC concentration in form of PNP increased up to 60% (see Table 3.1). This new feeding strategy also resulted in an increase of the percentage of reaction phase time in endogenous conditions up to 62%.

A DO profile for one SBR cycle operated with the new feeding strategy is shown in Figure 3.3. The arrows indicate the partial feeds and the instantaneous decrease of the DO concentration after each partial filling (distributed additions of PNP) which corresponded to the exogenous period of organic substrate consumption. This exogenous period finished when the DO concentration increased again until reaching similar levels to those preceding the PNP addition. This last period corresponded to endogenous conditions, i.e. without external substrate availability. The success of this feeding strategy for achieving a K-strategist population was tested with a stoichiometric and kinetic characterization of the activated sludge through modeling that will be presented in the next section.

Regarding the operational parameters of the SBR, the maximum PNP loading rate applied (0.26 g PNP g^{-1} VSS d^{-1} in Table 3.2) was clearly lower than those achieved by Tomei et al. (2003), in a similar acclimated activated sludge operated without PNP limitation. However, the loading rate of this work was comparable to those achieved by other researchers in biofilm and granular reactors operated with PNP limitation (Bhatti et al., 2002; Yi et al., 2006). They obtained complete PNP degradations with PNP loading rates up to 0.165 g PNP g^{-1} VSS d^{-1} and 0.11 g PNP g^{-1} VSS d^{-1} respectively, whereas the PNP loading rate obtained in this work on the last period (days 181 to

350) without any instability problem was 0.14 g PNP g^{-1} VSS d^{-1}. On the other hand, the inoculated biomass concentration (4600 mg VSS L^{-1}) showed a continuous decrease and reached a steady-state (1300 mg VSS L^{-1}) around day 200 (Table 3.2). This decrease was due to the biomass acclimation to successive increases in the percentage of PNP in the influent. A similar trend for the biomass concentration was found in an acclimation study on PNP with immobilized biomass (Xing et al., 1999).

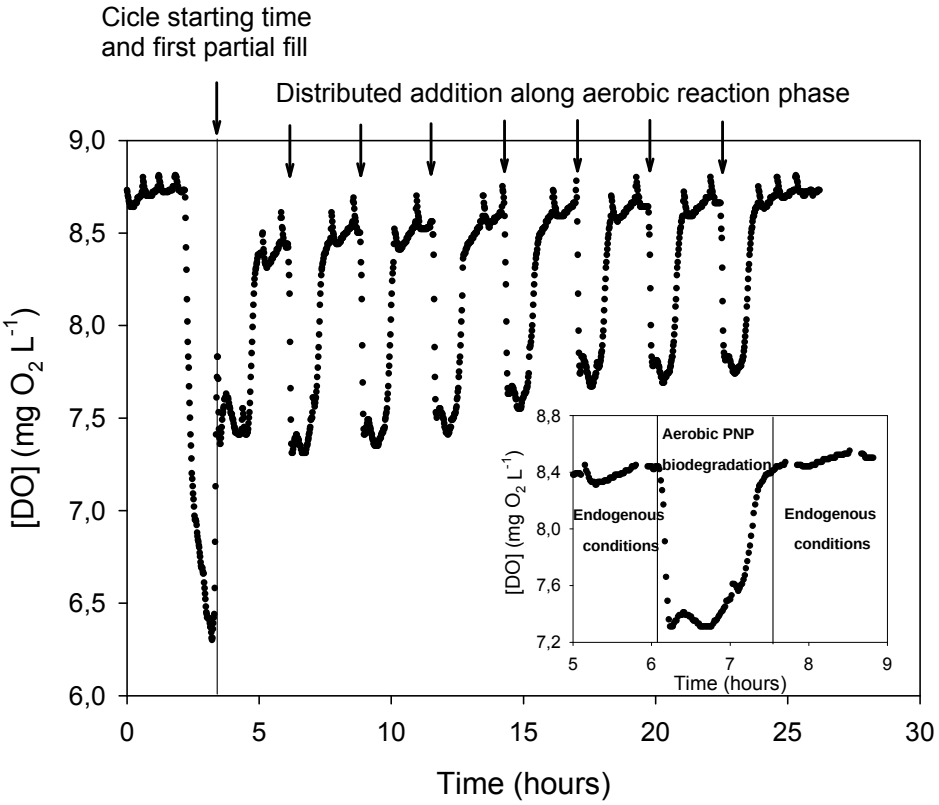


Figure 3.3. DO profile for one cycle in the period with distributed addition of PNP along the aerobic reaction phase. This feeding strategy was applied to the last 169 days (from day 181 to day 350) of SBR operation with an influent with 60% of TOC as PNP.

3.3.1.1. Kinetic characterization of the PNP-degrading activated sludge through modelling

On day 300 of SBR operation, a stoichiometric and kinetic characterization of the PNP-degrading activated sludge was carried out. This characterization consisted of (i) determination of the growth yield coefficient; (ii) calibration of the mathematical kinetic model described in section 3.2.4

using a batch respirometric test; and (iii) validation of the model with a different batch respirometric test.

3.3.1.2. Determination of the growth yield coefficient

One of the parameters needed for the calibration of this model was the growth yield coefficient for PNP biodegradation ($Y_{X/PNP}$). The option of using a literature value was discarded due to the wide range of $Y_{X/PNP}$ values reported (0.27 (Bhatti et al., 2002); 0.43-0.50 (Löser et al., 1998); 0.56-0.62 (Tomei et al., 2003); all values in mg COD mg^{-1} PNP). Consequently, $Y_{X/PNP}$ was determined from our own set of respirometric experiments. This set consisted of several batch tests at different and known initial PNP concentrations. $Y_{X/PNP}$ was obtained by plotting the total oxygen consumption (OC) versus the initial PNP concentration in each respirometry data set (Figure 3.4). The OC was calculated as the area under the exogenous OUR curve. The regression slope corresponded to the oxygen demand per unit of PNP ($OC = (1-Y_{X/PNP})\cdot[PNP]$) and allowed the calculation of $Y_{X/PNP}$. The obtained value was at the lower end of the range reported in the literature: $Y_{X/PNP} = 0.28 \pm 0.05$ mg COD mg^{-1} PNP (or $Y_{X/PNP} = 0.20 \pm 0.04$ mg VSS mg^{-1} PNP considering biomass as $C_5H_7NO_2$).

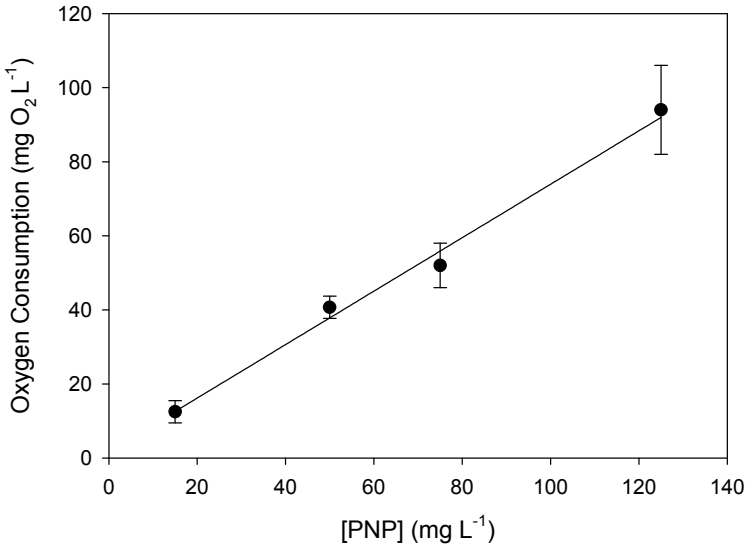


Figure 3.4. Growth yield coefficient ($Y_{x/PNP}$) determination: oxygen consumption as a function of the initial PNP concentration.

3.3.1.3. Calibration of the model

To calibrate the model, a set of experimental data from a respirometric batch test (Figure 3.5a) was used. Experimental data include time-course off-line PNP measurements as well as, SOUR values recorded on-line from the DO sensor measurements. The experimental conditions were: an initial PNP concentration of 125 mg PNP L^{-1} as the sole organic carbon source, pH 7.5 and temperature of 26 ºC. From Figure 3.5a, an initial lag phase lasting about 30 min can be noted. This effect was repeatedly observed in all the tests performed for the growth yield determination (data not shown). This behaviour was likely caused by inhibition of the microbial population due to the PNP addition, as the concentrations used in the tests were higher than those used in the SBR feeding strategy (Heitkamp et al., 1990; Tomei et al., 2008). Taking into account the complexity of the possible phenomena occurring in the lag phase, the initial data points of PNP concentration and SOUR were not taken into account for the model calibration. After this lag phase, a slow increase of the SOUR values was observed and, after reaching a maximum, there was a fast decay which coincided with the total depletion of PNP showing a trend that can be described with Haldane's kinetic model (Jubany et al., 2005).

It was assumed that the total biomass in the respirometer remained constant during the experiments. This is a reasonable assumption since the respirometer measurements lasted from three to six hours, depending on initial PNP concentration and including the time to adjust respirometry conditions. In this very short period of time no significant biomass growth was observed. Haldane's model correctly described the experimental data of SOUR and PNP concentration as can be seen in Figure 3.5.a, in particular for the final values of SOUR that are the most sensitive data when calibrating the K_s and the most important data to classify the microbial population as r- or K-strategist.

- *Parameter estimation*

For the estimation of the kinetic parameters vector, $\vec{\theta}$=(k_{max}, K_s, K_i) the

following weighted objective function was used (i.e. Eq. (3.5)) applied for this specific experiment, substituting $\vec{y}$ by the specific data sets used):

$$G\left(\vec{\theta}\right) = w_1 \cdot norm\left(\left[PNP\right]^{\exp} - \left[PNP\right]^{model}\left(\vec{\theta}\right)\right) + w_2 \cdot norm\left(SOUR^{\exp} - SOUR^{model}\left(\vec{\theta}\right)\right) + w_3 \cdot norm\left(SOUR_{fd}^{\exp} - SOUR_{fd}^{model}\left(\vec{\theta}\right)\right) \quad \text{Equation 3.8}$$

where the array $\vec{\theta} = (\theta_1, \theta_2, \theta_3)$ is the vector of model parameters, with $\theta_1 = k_{\max}$; $\theta_2 = K_s$; $\theta_3 = K_i$; w_i are the weighting coefficients used for each one of the data sets included in the objective function; $norm_i$ is the Euclidean norm of a vector, defined as $norm\left(\vec{X}\right) = \sqrt{\sum_{i=1}^{n} x_i^2}$ for each data set included in the objective function; $[PNP]^{\exp}$ and $SOUR^{\exp}$ are the vectors of the experimental data of each data set, $[PNP]^{model}$ and $SOUR^{model}$ are the vectors containing the modeling results evaluated for a specific set of parameters $\vec{\theta}$.

Therefore, both, the values of time-course PNP concentration as well as SOUR values were simultaneously optimized to reduce the uncertainty of the estimated parameters. As can be observed in Eq. (3.8) the SOUR set of data was divided in two different arrays, one including all data points with the exception of those in the $SOUR_{fd}^{\exp}$ which corresponds to the fast decrease of SOUR when PNP is being depleted at the end of the batch respirometric test. Splitting the SOUR data set into two subsets was necessary to independently vary the weighting coefficients (w_i) in Eq. (3.8). The steep slope in the fast decrease set is strongly dependent on the value of K_s, and therefore an incorrect design of the objective function (Eq. (3.8)) was leading, unavoidably, to a lack of satisfactory description of the $SOUR_{fd}^{\exp}$ by the mathematical model (i.e. a wrong value for the K_s coefficient).

To obtain the weighting coefficients in Eq. (3.8), w_1 was set to 1 and w_2 and w_3 were estimated through optimization (see section 3.2.5) to minimize the standard error of K_s (Eq. (3.6)). The results obtained were w_2 = 0.9877 and w_3 = 49.67.

The kinetic parameters obtained in the calibration and their confidence intervals are shown in Table 3.3. As expected, the parameter with a highest uncertainty is K_s.

Table 3.3. Kinetic parameters obtained in the model calibration with a biomass acclimatized to PNP. Estimation of the accuracy of the parameters is given for a confidence level of 95%.

	k_{max} (mg PNP mg^{-1} COD d^{-1})	K_s (mg PNP L^{-1})	K_i (mg PNP L^{-1})
Value	1.02	1.6	54
Confidence interval	[1.01-1.04]	[1.0-2.7]	[52-55]

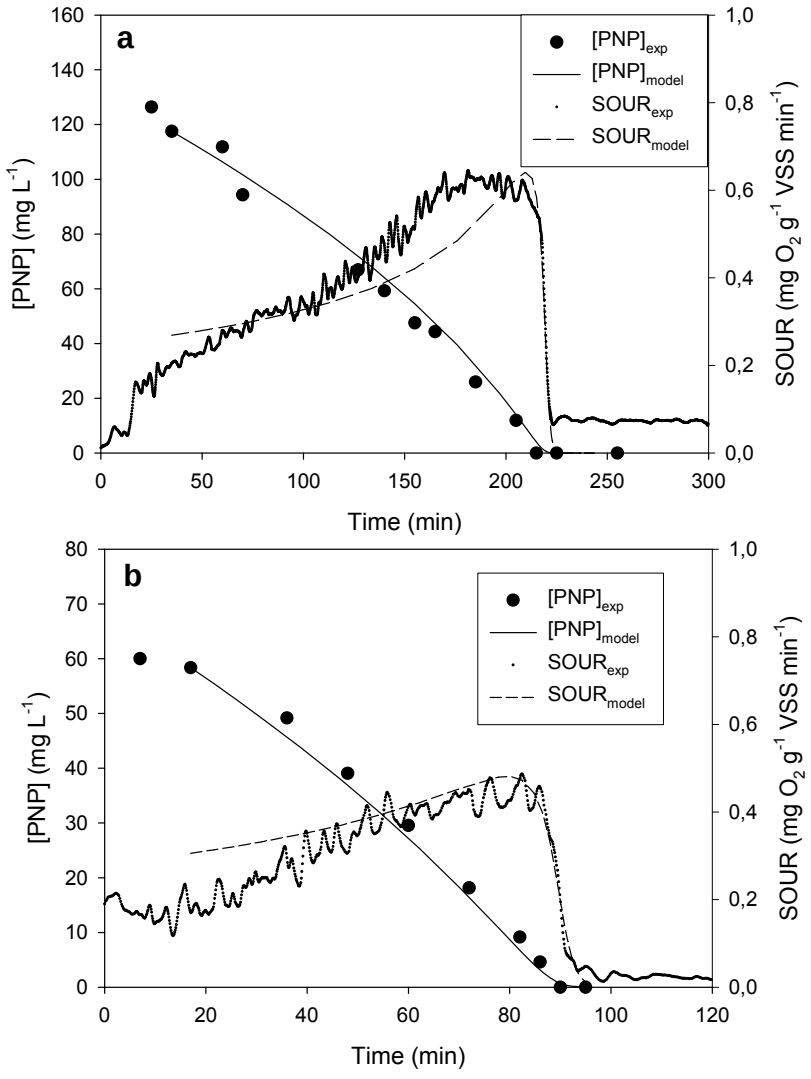


Figure 3.5. (a) Calibration of the mathematical model with the experimental data obtained in a respirometric test with an initial concentration of 125 mg PNP L^{-1} (pH = 7.5 and T = 26 ºC). (b) Validation of the mathematical model with the experimental data obtained in a respirometric test with an initial concentration of 60 mg PNP L^{-1} (pH = 7.5 and T = 26 ºC).

3.3.2.3. Validation of the model

The kinetic coefficients obtained in the calibration were validated with another respirometric batch test performed with an initial concentration of 60 mg PNP L^{-1}, as the sole organic carbon source. The validation was performed maintaining the calibrated values of K_i and K_s and estimating, through optimization, the value of k_{max} with Eq. (3.8) and the weighting coefficients (w_i) already determined in the calibration. In ASM-based models as the one described in this study, the biomass is considered as a particulate compound (*X*) that grows in an aerobic environment (with a growing process rate proportional to the maximum specific growth rate (μ_{max})) by consuming a soluble substrate as carbon and energy source (with a consuming process rate proportional to the maximum specific degradation rate (k_{max})). The biomass concentration (*X*) is experimentally quantified as the volatile suspended solids concentration in the bioreactor [*VSS*], but is well know that only a fraction of the total *VSS* concentration is active biomass growing and consuming substrate at a rate defined by the kinetic expressions considered in the model. This fraction is named active biomass fraction (f_x). Consequently, the biomass concentration considered in the ASM-based models is $X = f_x \cdot [VSS]$. The fraction of active biomass depends on the history of the biomass and its value might change between two different experiments (Jubany et al., 2005; Lindblom et al., 2009). The active biomass fraction is usually considered as a lumped parameter included in the term $k_{\max} \cdot X$ (Petersen et al., 2003). If an experimental [*VSS*] is measured and considered for each modelling experiment (calibration and validation), any difference in the active biomass fraction (f_x) between both experiments will result in a variation of k_{max} value. The k_{max} obtained in the validation was 0.91 mg PNP mg^{-1} COD d^{-1}. Figure 3.5b shows the experimental and simulated data for the validation. As can be seen, the model correctly described the trend of the kinetics for PNP consumption after the initial lag phase as well as the SOUR profile.

Other studies also successfully modeled similar systems but only fitting the PNP consumption profile (Tomei et al., 2003) or the OUR profile (Tomei et al., 2004), none of them simultaneously fitted both profiles. The model

predictions shown in Figure 3.5 demonstrate that the model successfully describes the PNP degradation even when using an activated sludge acclimated to degrade PNP with another biodegradable co-substrate and taking into consideration respirometric tests carried out with PNP as the sole organic carbon source.

3.3.2.4. Assessment of the obtained kinetic parameters

The PNP-degrading mixed populations reported in the literature were usually developed in reactors operating without PNP limitation or with short periods of PNP limitation (Tomei et al., 2003; Tomei and Annesini, 2005; Yi et al., 2006). For example, Tomei et al, (2003) reported that the effluent of their reactor had a stable PNP concentration of 0.5 to 1.0 mg L^{-1} during more than 100 days. These non-limiting conditions resulted in the development of r-strategist populations with high K_s and high maximum activities (Table 3.4). The K_s obtained for the PNP-degrading activated sludge in this work is one order of magnitude lower than the bibliographic K_s values for mixed populations (Table 3.4). Moreover, the k_{max} measured in the present work (reported in mg PNP mg^{-1} VSS d^{-1}) was lower than that reported in most other references (Table 3.4). Both differences allowed the classification of the microbial population developed in this work as K-strategist (Blackburne et al., 2007; Jih et al., 2008; Schramm et al., 1999) and reflect the success of the feeding strategy applied.

Table 3.4. Kinetic parameters for aerobic PNP degradation reported in the literature.

K_s (mg PNP L^{-1})	k_{max} (g PNP g^{-1}VSS d^{-1})	K_i (mg PNP L^{-1})	pH	T (ºC)	Culture	Reference
55	6.99	15	7-8	20	Mixed (Activated sludge)	Tomei et al, (2005)
17.6	3.3-8.4	30.7	7-8	20	Mixed (Activated sludge)	Tomei et al, (2004)
20	6.68	12	6.5-8.5	20	Mixed (Activated sludge)	Tomei et al, (2003)
17.9	0.88	89.7	7.1	-	Mixed (Granular sludge)	Yi et al, (2006)
0.145	-	-	7	30	Pure (*Pseudomonas putida*)	Löser et al, (1998)
39-45	-	-	-	30	Pure (*Arthrobacter protophormiae* and *Ralstonia sp.*)	Bhushan et al, (2000)
0.09	-	1.66	-	30	Pure (*Bacillus sphaericus*)	Kadiyala et al, (1998)
1.6	1.45	54	7.5	26	Mixed (Activated sludge)	This work

The only references reporting microbial populations with K_s significantly lower than those obtained in this work corresponded to pure cultures developed with strains isolated from contaminated environments and classified as PNP-degraders (Table 3.4). These populations were cultivated under PNP limitation (Kadiyala et al., 1998; Löser et al., 1998), which confirms the feeding strategy for obtaining a K-strategist culture. However, the protocols reported in these references to develop a PNP-degrading population could not be applied without difficulties at full-scale as they were carried out using sterile media and resulted in low biomass concentrations.

Another important feature of the kinetic parameters obtained for the PNP-degrading population of this study is the K_i value. The obtained inhibition constant is higher than the values reported in most of the bibliographic references (Table 3.4). This means that the acclimated activated sludge of this work was more adapted to PNP inhibition than the rest of reported cultures. This trend could be very useful since it was demonstrated that the inhibition by PNP can cause process failure (Bhatti et al., 2002; Ray et al., 1999).

3.3.3. Indirect evidence of the PNP oxidation pathway

Two alternative pathways exist for aerobic PNP biodegradation (Ye et al., 2004). In the first, HQ is formed from PNP with simultaneous release of nitrite. Subsequently, HQ is oxidised to β-ketoadiapate (Spain and Gibson, 1991). In the second pathway, 4-NC is formed from PNP followed by a transformation into 1,2,4-BT with a related release of nitrite. Afterwards, 1,2,4-BT is oxidised to β-ketoadiapate (Jain et al., 1994).

The rapid consumption of an intermediate as the sole carbon source can be used as an aid in determining the oxidation pathway, provided that intermediate compound can readily enter the cells (Liu et al., 2007; Wan et al., 2007; Zhang et al., 2009). For instance, Liu et al, (2007) found that a pure strain of a PNP-degrading bacterium (*Stenotrophomonas* sp. LZ-1) was able to consume HQ as the sole carbon source but was unable to consume 4-NC and therefore concluded that the metabolic pathway to be via HQ. On the

other hand, Wan et al, (2007) and Zhang et al, (2009) found that other pure strains of PNP-degrading bacteria (*Achromobacter xylosoxidans* Ns and *Rhodococcus* sp. CN6, respectively) were able to consume 4-NC as the sole carbon source. Therefore, according to them, the metabolic pathway involved 4-NC.

In this context, two respirometric batch tests were carried out with the main intermediates of each pathway (HQ and 4-NC) as the sole carbon source to try to elucidate the metabolic pathway involved in the PNP degradation. Each test consisted in the addition of (i) the intermediate as the sole carbon source, and (ii) simultaneous addition of the intermediate and PNP. Both tested intermediates were completely consumed by the enriched K-strategist microbial population when they were added as the sole carbon source (Figure 3.6). Moreover, the oxygen consumption measured during these tests and the consumption of the intermediate occurred simultaneously. Nevertheless, the intermediate specific consumption rate varied significantly depending on the substance: 67.2 mg L^{-1} h^{-1} for HQ and 2.8 mg L^{-1} h^{-1} for 4-NC. No significant variations in the consumption rates were detected when PNP and intermediate were added simultaneously (Figure 3.6). However, there was an important difference between 4-NC and HQ. The 4-NC was consumed only when PNP was completely depleted (Figure 3.6b) while HQ was depleted faster than PNP (Figure 3.6a). On the other hand, the oxygen consumption measured in the simultaneous biodegradation of PNP and the intermediate was appreciably higher than that obtained with the intermediate as the sole carbon source due to the oxygen consumption associated with PNP biodegradation.

The consumption of both intermediates (HQ and 4-NC) shown in Figure 3.6 excludes the possibility of assigning one of the reported metabolic pathways of PNP degradation as unique. Several hypotheses could be postulated for interpreting the results: (i) The PNP-degrading activated sludge might be a mixed culture formed by several microbial species. Some bacteria would degrade PNP via 4-NC and others via HQ. (ii) The PNP-degrading activated sludge would mainly follow the HQ pathway but would also consume 4-NC. For instance, (Chauhan et al., 2000) found that *Arthrobacter*

protophormiae strain RKJ100 aerobically degraded PNP via HQ but it was also capable of utilizing 4-NC as the sole carbon source. (iii) The degradation of PNP would follow a third, completely different pathway with both 4-NC and HQ as intermediates. In this context, (Qiu et al., 2006) found that *Ochrobactrum* sp. B2 aerobically degraded methyl parathion with PNP, 4-NC and HQ as intermediates. Further studies are required to confirm these hypotheses.

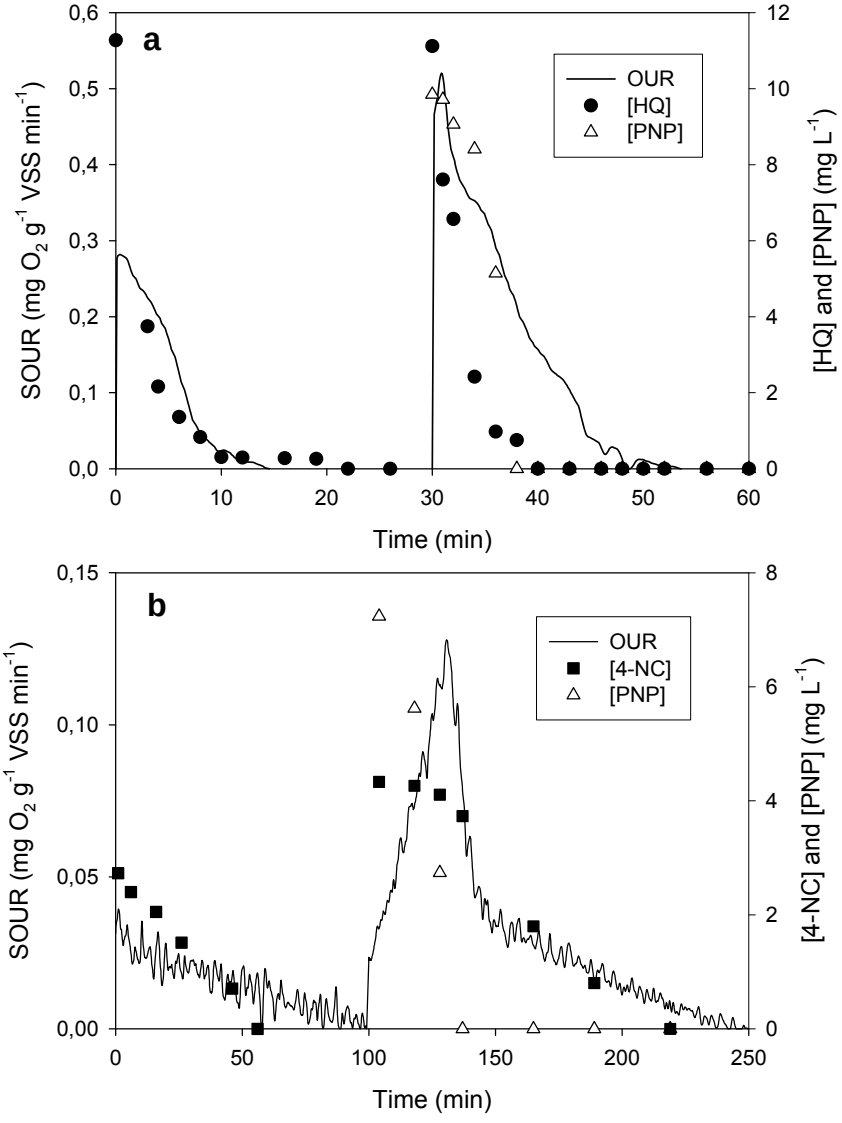


Figure 3.6 (a) Respirometric tests with HQ as the sole carbon source and simultaneous addition of an HQ and PNP as carbon sources (pH = 7.5 and T = 26 ºC). (b) Respirometric tests with 4-NC as the sole carbon source and simultaneous addition of a 4-NC and PNP as carbon sources (pH = 7.5 and T = 26 ºC).

3.4. Conclusions

In an SBR, a feeding strategy was implemented for the enrichment of a K-strategist PNP-degrading activated sludge using conventional activated sludge from a municipal WWTP (i.e. non-acclimated biomass) as inoculum. The strategy was based on the distribution of the PNP addition along the aerobic reaction phase imposing endogenous conditions in 62% of the total reaction phase time of the SBR.

Ninety-seven per cent of TOC removal and 100% of PNP elimination were achieved in the course of the whole operating period; with specific TOC loading rates between 0.13 to 0.38 g TOC g^{-1} VSS d^{-1} and with a maximum specific PNP loading rate of 0.26 g PNP g^{-1} VSS d^{-1}. No metabolic intermediates were detected.

A mathematical model successfully describing the PNP degradation and the oxygen consumption was calibrated and validated using respirometric batch tests. The values obtained for the affinity constant for PNP (K_s) and the maximum specific PNP removal rate (k_{max}) allowed the biomass to be classified as a K-strategist and to corroborate the success of the applied feeding strategy. Moreover, the value obtained for the substrate inhibition constant (K_i) is higher than that reported by most bibliographic references. This means that the acclimated activated sludge of this work was more adapted to PNP inhibition than the remainder of the reported cultures.

3.5. References

Andrews, J.F. 1968. A mathematical model for continuous culture of microorganisms utilizing inhibitory substrates. *Biotechnology and Bioengineering*, 10(6), 707-723.

Andrews, J.H., Harris, R.F. 1986. r- and K-selection and microbial ecology. *Advances in Microbial Ecology*, 9, 99-147.

Antileo, C., Aspe, E., Urrutia, H., Zaror, C., Roeckel, M. 2002. Nitrifying biomass acclimation to high ammonia concentration. *Journal of Environmental Engineering-Asce*, 128(4), 367-375.

ATSDR. 1992. Toxicological Profile for nitrophenols: 2-Nitrophenol and 4-Nitrophenol. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA., (Ed.) A.f.T.S.a.D. Registry.

Beale, E. 1960. Confidence-regions in non-linear estimation. *Journal of the Royal Statistical Society Series B-Statistical Methodology*, 22(1), 41-88.

Bhargava, S., Tardio, J., Prasad, J., Foger, K., Akolekar, D., Grocott, S. 2006. Wet oxidation and catalytic wet oxidation. *Industrial & Engineering Chemistry Research*, 45(4), 1221-1258.

Bhatti, Z.I., Toda, H., Furukawa, K. 2002. p-Nitrophenol degradation by activated sludge attached on nonwovens. *Water Research*, 36, 1135-1142.

Bhushan, B., Chauhan, A., Samanta, S., Jain, R. 2000. Kinetics of biodegradation of p-nitrophenol by different bacteria. *Biochemical and Biophysical Research Communications*, 274(3), 626-630.

Blackburne, R., Vadivelu, V., Yuan, Z., Keller, J. 2007. Kinetic characterisation of an enriched Nitrospira culture with comparison to Nitrobacter. *Water Research*, 41(14), 3033-3042.

Chauhan, A., Chakraborti, A., Jain, R. 2000. Plasmid-encoded degradation of p-nitrophenol and 4-nitrocatechol by Arthrobacter protophormiae. *Biochemical and Biophysical Research Communications*, 270(3), 733-740.

Dai, Q., Lei, L., Zhang, X. 2008. Enhanced degradation of organic wastewater containing p-nitrophenol by a novel wet electrocatalytic oxidation process: Parameter optimization and degradation mechanism. *Separation and Purification Technology*, 61(2), 123-129.

Di Mattia, E., Grego, S., Cacciari, I. 2002. Eco-physiological characterization of soil bacterial populations in different states of growth. *Microbial Ecology*, 43(1), 34-43.

Dochain, D., Vanrolleghem, P.A. 2001. *Dynamical Modelling and Estimation in Wastewater Treatment Processes*. IWA Publishing, London.

Draper, N.R., Smith, H. 1998. *Applied Regression Analysis, 3th edition*. Wiley-Interscience, USA.

Dytczak, M., Londry, K., Oleszkiewicz, J. 2008. Activated sludge operational regime has significant impact on the type of nitrifying community and its nitrification rates. *Water Research*, 42(8-9), 2320-2328.

EPA. 2009. EPA Integrated Risk Information System: *p*-Nitrophenol. Available from: http://www.epa.gov/ncea/iris/subst/0484.htm. Last time consulted: 26/04/2012.

Gentry, T., Rensing, C., Pepper, I. 2004. New approaches for bioaugmentation as a remediation technology. *Critical Reviews in Environmental Science and Technology*, 34(5), 447-494.

Ginige, M., Carvalho, G., Keller, J., Blackall, L. 2007. Eco-physiological characterization of fluorescence in situ hybridization probe-targeted denitrifiers in activated sludge using culture-independent methods. *Letters in Applied Microbiology*, 44(4), 399-405.

Heitkamp, M.A., Camel, V., Reuter, T.J., Adams, W.J. 1990. Biodegradation of para-nitrophenol in an aqueous waste stream by immobilized bacteria. *Applied and Environmental Microbiology*, 56(10), 2967-2973.

Henze, M., Gujer, W., Mino, T., Van Loosdretch, M.C.M. 2000. Activated sludge models ASM1, ASM2 ASM2d and ASM3: Scientific and technical report No.9. *IWA Task Group on Mathematical Modelling for Design and Operation of Biological Wastewater Treatment*.

Hu, X., Li, A., Fan, J., Deng, C., Zhang, Q. 2008. Biotreatment of p-nitrophenol and nitrobenzene in mixed wastewater through selective bioaugmentation. *Bioresource Technology*, 99(10), 4529-4533.

Jain, R.K., Dreisbach, J.H., Spain, J.C. 1994. Biodegradation of *p*-nitrophenol via 1,2,4-benzenetriol by an *Arthrobacter* sp. *Applied and Environmental Microbiology*, 60(8), 3030-3032.

Jih, C., Huang, J., Lin, H., Chou, H. 2008. Comparative kinetic behavior of nitrifiers with different growth environments. *Bioresource Technology*, 99(9), 3484-3490.

Jubany, I., Baeza, J., Carrera, J., Lafuente, J. 2005. Respirometric calibration and validation of a biological nitrite oxidation model including biomass growth and substrate inhibition. *Water Research*, 39(18), 4574-4584.

Juteau, P., Larocque, R., Rho, D., LeDuy, A. 1999. Analysis of the relative abundance of different types of bacteria capable of toluene degradation in a compost biofilter. *Applied Microbiology and Biotechnology*, 52(6), 863-868.

Kadiyala, V., Smets, B., Chandran, K., Spain, J. 1998. High affinity p-nitrophenol oxidation by Bacillus sphaericus JS905. *Fems Microbiology Letters*, 166(1), 115-120.

Labana, S., Pandey, G., Paul, D., Sharma, N., Basu, A., Jain, R. 2005a. Pot and field studies on bioremediation of p-nitrophenol contaminated soil using Arthrobacter protophormiae RKJ100. *Environmental Science & Technology*, 39(9), 3330-3337.

Labana, S., Singh, O., Basu, A., Pandey, G., Jain, R. 2005b. A microcosm study on bioremediation of p-nitrophenol-contaminated soil using Arthrobacter protophormiae RKJ100. *Applied Microbiology and Biotechnology*, 68(3), 417-424.

Lindblom, E., Press-Kristensen, K., Vanrolleghem, P., Mikkelsen, P., Henze, M. 2009. Dynamic experiments with high bisphenol-A concentrations modelled with an ASM model extended to include a separate XOC degrading microorganism. *Water Research*, 43(13), 3169-3176.

Liu, Z., Yang, C., Qiao, C. 2007. Biodegradation of p-nitrophenol and 4-chlorophenol by Stenotrophomonas sp. *Fems Microbiology Letters*, 277(2), 150-156.

Loser, C., Oubelli, M., Hertel, T. 1998. Growth kinetics of the 4-nitrophenol degrading strain Pseudomonas putida PNP1. *Acta Biotechnologica*, 18(1), 29-41.

Löser, C., Oubelli, M., Hertel, T. 1998. Growth kinetics of the 4-nitrophenol degrading strain Pseudomonas putida PNP1. *Acta Biotechnologica*, 18(1), 29-41.

Marsili-Libelli, S., Giusti, E. 2008. Water quality modelling for small river basins. *Environmental Modelling & Software*, 23(4), 451-463.

Marsili-Libelli, S., Guerrizio, S., Checchi, N. 2003. Confidence regions of estimated parameters for ecological systems. *Ecological Modelling*, 165(2-3), 127-146.

MATLAB. 1999. Optimization Toolbox: User's guide. Version 2 (release 11). *The MathWorks, Natick, MA, USA*.

OECD. 2008. The 2004 Organisation for Economic Co-operation and Development (OECD) List of High Production Volume Chemicals. http://www.oecd.org/dataoecd/55/38/33883530.pdf. Last time consulted: 26/04/2011, (Ed.) O.f.E.C.-o.a. Development.

Petersen, B. 2000. Identifiability and Optimal Experimental Design of Activated Sludge Models. PhD thesis, Ghent University. Ghent, Belgium. Available from: http://biomath.ugent.be/publications/download/petersenbritta_phd.pdf. Last time consulted: 26/04/2012, pp. .

Petersen, B., Gernaey, K., Devisscher, M., Dochain, D., Vanrolleghem, P. 2003. A simplified method to assess structurally identifiable parameters in Monod-based activated sludge models. *Water Research*, 37(12), 2893-2904.

Qiu, X., Bai, W., Zhong, Q., Li, M., He, F., Li, B. 2006. Isolation and characterization of a bacterial strain of the genus Ochrobactrum with methyl parathion mineralizing activity. *Journal of Applied Microbiology*, 101(5), 986-994.

Ray, P., Oubelli, M., Löser, C. 1999. Aerobic 4-nitrophenol degradation by microorganisms fixed in a continuously working aerated solid-bed reactor. *Applied Microbiology and Biotechnology*, 51(2), 284-290.

Razo-Flores, E.a., Donlon, B., Lettinga, G., Field, J.A. 1997. Biotransformation and biodegradation of N-substituted aromatics in methanogenic granular sludge. 20(3-4), 525 - 538.

Schramm, A., de Beer, D., van den Heuvel, J., Ottengraf, S., Amann, R. 1999. Microscale distribution of populations and activities of Nitrosospira and Nitrospira spp. along a macroscale gradient in a nitrifying bioreactor: Quantification by in situ hybridization and the use of microsensors. *Applied and Environmental Microbiology*, 65(8), 3690-3696.

Singer, A., van der Gast, C., Thompson, I. 2005. Perspectives and vision for strain selection in bioaugmentation. *Trends in Biotechnology*, 23(2), 74-77.

Spain, J., Gibson, D. 1991. Pathway for biodegradation of para-nitrophenol in a Moraxella SP. *Applied and Environmental Microbiology*, 57(3), 812-819.

Spanjers, H., Varolleghem, P., Olsson, G., Dold, P.I. 1998. *Respirometry in control of the activated sludge process: principles.* International Association on Water Quality, London, England.

Suárez-Ojeda, M., Guisasola, A., Baeza, J., Fabregat, A., Stuber, F., Fortuny, A., Font, J., Carrera, J. 2007. Integrated catalytic wet air oxidation and aerobic biological treatment in a municipal WWTP of a high-strength o-cresol wastewater. *Chemosphere*, 66(11), 2096-2105.

Tomei, M., Annesini, M., Luberti, R., Cento, G., Senia, A. 2003. Kinetics of 4-nitrophenol biodegradation in a sequencing batch reactor. *Water Research*, 37(16), 3803-3814.

Tomei, M., Annesini, M., Rita, S., Daugulis, A. 2008. Biodegradation of 4-Nitrophenol in a two-phase sequencing batch reactor: concept demonstration, kinetics and modelling. *APPLIED MICROBIOLOGY AND BIOTECHNOLOGY*, 80(6), 1105-1112.

Tomei, M.C., Annesini, M.C. 2005. 4-Nitrophenol Biodegradation in a Sequencing Batch Reactor Operating with Aerobic − Anoxic Cycles. *Environmental Science Technology*, 39, 5059-5065.

Tomei, M.C., Annesini, M.C., Bussoleti, S. 2004. 4-nitrophenol biodegradation in a sequencing batch reactor: kinetic study and effect of filling time. *Water Research*, 38, 375-384.

Wan, N., Gu, J.-D., Yan, Y. 2007. Degradation of p-nitrophenol by Achromobacter xiloxidans Ns isolated from wetland sediment. *International Biodeteroration & Biodegradation*, 59, 90-96.

Xing, X., Inoue, T., Tanji, Y., Unno, H. 1999. Enhanced microbial adaptation to p-nitrophenol using activated sludge retained in porous carrier particles and, simultaneous removal of nitrite released from degradation of p-nitrophenol. *Journal of Bioscience and Bioengineering*, 87(3), 372-377.

Ye, J., Singh, A., Ward, O. 2004. Biodegradation of nitroaromatics and other nitrogen-containing xenobiotics. *World Journal of Microbiology & Biotechnology*, 20(2), 117-135.

Yi, S., Zhuang, W.-Q., Wu, B., Tiong-Lee Tay, S., Tay, J.-H. 2006. Biodegradation of p-nitrophenol by aerobic granules in a sequencing batch reactor. *Environmental Science & Technology*, 40, 2396-2401.

Zhang, J., Sun, Z., Li, Y., Peng, X., Li, W., Yan, Y. 2009. Biodegradation of p-nitrophenol by rhodococcus sp. CN6 with high cell surface hydrophobicity. *Journal of Hazardous Materials*, 163, 723-728.

CHAPTER 4

Bioaugmentation for treating transient or continuous *p*-nitrophenol shock loads in an aerobic sequencing batch reactor

The contents of this chapter have been sent for publication to the Bioresource Technology journal.

Abstract

In this study, bioaugmentation with an enriched microbial population was applied in an aerobic sequencing batch reactor (SBR) receiving transient or continuous shock loads of *p*-nitrophenol (PNP). The effect of the amount of enriched microbial population added for bioaugmentation was assessed by using two different dosages (2 or 5% w/w of the total biomass in the seeded SBR). In both cases, total PNP removal was achieved during the transient PNP shock load occurring after bioaugmenting the SBR. However, after a long PNP starvation period the only experiment still showing total PNP removal during a second PNP shock load was the one where a dosage of 5% w/w was applied. Moreover, fluorescence in-situ hybridisation (FISH) was used to follow the microbial population evolution during experiments in order to explain the obtained results. The results suggested that the dosage is a key factor for the implementation of a successful bioaugmentation strategy. In addition, the performance of a bioaugmented SBR receiving a continuous PNP shock load was enhanced when compared to a non-bioaugmented SBR.

4.1. Introduction

Biological treatments are the preferred option in terms of economical costs and ecological footprint, if compared to chemical or physical treatments for treating industrial wastewaters containing organic pollutants. Usually, biological treatments are capable of properly removing organic carbon and

nutrients, but in the case of some industrial wastewaters containing inhibitory and recalcitrant substances, the operation of biological reactors could be problematic, and any perturbation may result in a poor performance of the treatment (Kulkarni and Chaudhari, 2007; Quan et al., 2004).

The operation mode in a great deal of industries operate is discontinuous, since they might have fluctuations in the flow according to demand or even produce different products within the same plant. This scenario might be difficult to handle a wastewater treatment plant (WWTP) because the biological reactor could be forced to receive transient or continuous shock loads of a particular contaminant (Sipma et al., 2010). In these cases, the bioaugmentation of a biological reactor could be indicated as a viable treatment strategy to deal with this problem (Ma et al., 2009; Schauer-Gimenez et al., 2010).

Bioaugmentation is defined as the introduction of a specific strain or a consortium of microorganisms with the aim of accelerating and enhancing the removal efficiency of recalcitrant and/or toxic compounds in polluted sites and bioreactors (El Fantroussi and Agathos, 2005; Vogel, 1996). Specialized microorganisms used in bioaugmentation may include indigenous or genetically modified strains. Sometimes, isolated strains are used for bioaugmentation of wastewater treatments (Nancharaiah et al., 2008; Olaniran et al., 2006; Park et al., 2008; Plangklang and Reungsang, 2011), but enriched mixed cultures are preferred because degradation of pollutants is more likely to be attained by active consortia of microorganisms, rather than from isolated strains (Chen et al., 2006; Mrozik and Piotrowska-Seget, 2010).

Successful cases of bioaugmentation using mixed consortia of bacteria have been reported for the improvement of the reactor start-up or for the recovery after shock loads in biological reactors (Bartroli et al., 2011; Guo et al., 2009; Ma et al., 2009). However, bioaugmentation is still not a widespread strategy due to the fact that the effects of bioaugmentation are difficult to predict and control (Chong et al., 1997; Yu et al., 2010). The success or failure of the bioaugmentation strategy depends on the ability of the introduced bacteria to survive and to display their activities in the mixed

culture (Mohan et al., 2005). The inability to retain the specialized bacteria is one of the most frequent problems in bioaugmentation; hence, characterization of the microbial population in the bioaugmented reactors may help understand this process and to optimize the operational conditions to achieve a successful bioaugmentation.

The objectives were: (i) to evaluate the benefits of bioaugmentation on PNP removal under transient or continuous shock loads and (ii) to study the persistence of the specialized PNP-degrading bacteria inside the bioaugmented reactor and its ability to degrade a transient PNP shock load after several sludge retention times (SRTs).

4.2. Materials and methods

4.2.1. Chemical compounds and analytical methods

All compounds used in this study were of analytical grade. PNP was in granular form with a purity >99% (provided by Sigma-Aldrich, Spain).

Total and volatile suspended solids (TSS and VSS) were determined using the procedures stated in Standard Methods for the examination of Water and Wastewater (APHA, 1998). Total organic carbon (TOC) was measured with an OI Analytical TOC Analyser (Model 1020A) equipped with a non-dispersive infrared (NDIR) detector and with a furnace maintained at 680ºC. PNP and metabolic intermediates concentrations were determined by High Performance Liquid Chromatography (HPLC) (UltiMate 3000, Dionex Corporation) using an Agilent Zorbax SB-C18 (4.6 mm x 100 mm, 3.5 µm) column and a UV detector set at 254 nm. Details of the HPLC method can be found elsewhere (Martín-Hernández et al., 2009).

4.2.2. Enriched PNP-degrading activated sludge and seeding reactor

PNP-degrading activated sludge used for bioaugmentation was taken from a 20 L SBR, namely the seeding reactor (SBR-A). SBR-A was treating a high-strength PNP wastewater during more than three years. This reactor was

operated to treat a high PNP concentration (380 mg L^{-1}) using glucose as co-substrate with a PNP:glucose ratio of 60:40 (in terms of TOC), whereas the specific loading rate was 0.14 g PNP g^{-1} VSS d^{-1} (Martín-Hernández et al., 2009). Temperature in the reactor was maintained with an on-off controller at 19-20ºC, pH was maintained at 7.5 by adding sodium bicarbonate to the feed and DO concentration remained between a range of 6.5-7.5 mg O_2 L^{-1}. The applied SRT was 20 days.

SBR-A was operated with a feeding strategy that allowed endogenous conditions (i.e. without external substrate availability) in up to 62% of the reaction phase; such conditions were necessary for achieving a K-strategist microbial population that is believed to have a more efficient performance, when used for bioaugmentation, to stand in front of transient shock loads are applied (Juteau et al., 1999; Labana et al., 2005). The PNP-degrading biomass was characterized and its kinetics was successfully described with a Haldane model with the following parameters (20ºC and pH=7.5): half-saturation coefficient (K_s) of 1.6 mg PNP L^{-1} and substrate inhibition constant (K_i) of 54 mg PNP L^{-1} (Martín-Hernández et al., 2009).

4.2.3. Wastewater description

Synthetic wastewater was used in this study with the following composition (in mg L^{-1}): $CaCl_2$ (93), $CO(NH_2)_2$ (13), KH_2PO_4 (41), $MgCl_2 \cdot 6H_2O$ (208), NH_4Cl (106), NaCl (185), yeast extract (2), $CuSO_4 \cdot 5H_2O$ (2) $FeSO_4$ (4), H_3BO_3 (0.02), $MnSO_4 \cdot H_2O$ (3), $ZnSO_4 \cdot 7H_2O$ (4). The organic carbon source of the synthetic wastewater was glucose (normal operation) or a mixture of glucose and PNP (operation with PNP shock loads) depending on the experimental period.

4.2.4. Experimental procedure for bioaugmentation experiments and operation of the seeded reactor

The SBR used in the bioaugmentation experiments was a 50 L stainless steel tank (seeded reactor, SBR-B), equipped with a mechanical stirrer, a

compressed air inlet, an air diffuser at the bottom of the reactor and a heating device. Feeding of SBR-B was made through a membrane pump while effluent draw was done using an electrical valve. SBR-B was provided with sensors to measure pH, temperature and DO, also with two valves for liquid sampling and a safety valve to prevent reactor flooding. SBR-B was monitored and controlled with a home-made program developed in LabView 8.2 (National Instruments).

The inoculation procedure was common for all experiments as follows: SBR-B was inoculated with an activated sludge from a municipal WWTP (i.e. non-acclimated biomass) located in Barcelona area and was fed with glucose as the sole carbon source. SBR-B was operated in cycles of 8 h, each one divided as follows: an aerobic reaction phase of 400 min (the filling phase was divided in 2 sub-phases along the reaction phase), a settling phase of 60 min, a draw phase of 15 min and an idle phase of 15 min. Hydraulic retention time (HRT) was 0.78 days whereas the purge was calculated to maintain a SRT of 20 days. The pH of the feed was adjusted to 7.5 by adding sodium bicarbonate in the feeding. Temperature on SBR-B was controlled at 20±1 ºC, while DO concentration was not controlled but maintained between in a range of 6.5-7.5 mg O_2 L^{-1}.

4.2.4.1. Transient shock loads

The performance of a bioaugmented reactor (SBR-B) under transient PNP shock loads was studied. Three experiments were carried out following the procedure described in Figure 4.1a control experiment without bioaugmentation and two bioaugmentation experiments with two different dosages of the enriched PNP-degrading activated sludge from SBR-A (corresponding to mass fractions of 2 and 5% w/w of the total biomass in SBR-B).

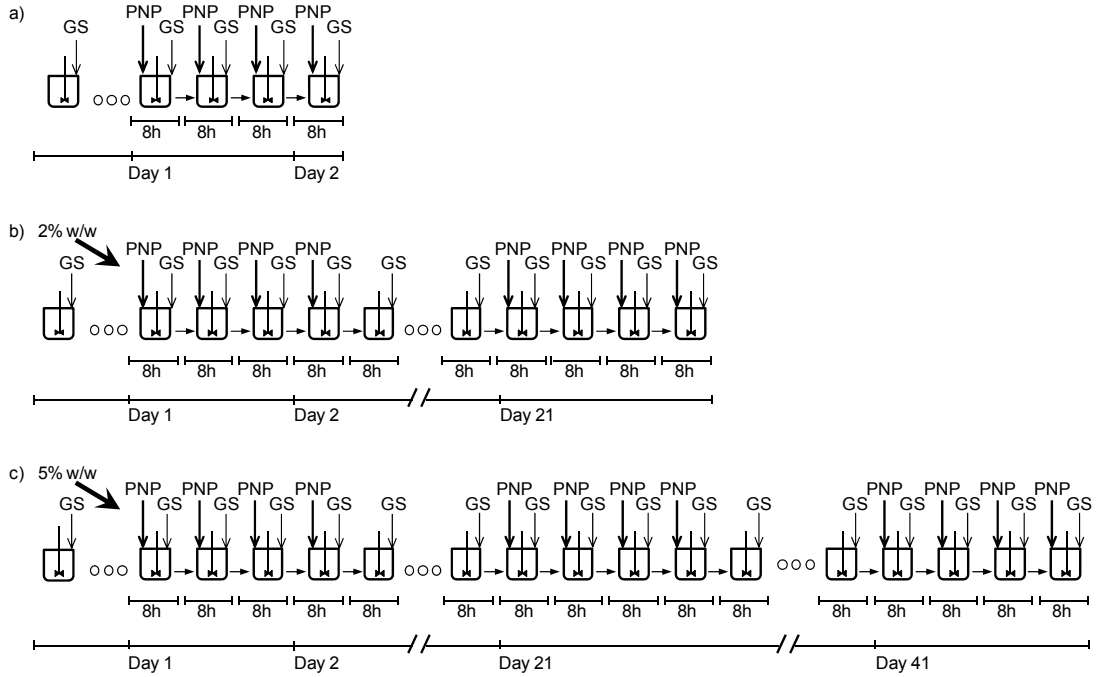


Figure 4.1. Experimental scheme for transient PNP shock loads experiments: a) without bioaugmentation, b) bioaugmentation with 2% w/w of the total biomass in the seeded reactor and c) bioaugmentation with 5% w/w of the total biomass in the seeded reactor. PNP = *p*-nitrophenol, GS = glucose.

4.2.4.2. Continuous shock load

The effect of a continuous PNP shock load was studied in SBR-B by adding continuously a feeding containing a mixture of glucose and PNP as the carbon source. A control experiment without bioaugmentation was also carried out. The PNP-degrading activated sludge dosage from SBR-A corresponded to a mass fraction of 5% w/w of the total biomass in SBR-B.

4.2.5. Fluorescence *in-situ* hybridization (FISH) coupled with confocal laser scanning microscopy (CLSM)

FISH coupled with CLSM was used to monitor and to quantify the PNP-degrading activated sludge from SBR-A in SBR-B during the bioaugmentation experiments. In a previous study (Suárez-Ojeda et al., 2011), the PNP-degrading activated sludge from SBR-A was identified as a mixed culture where the microorganisms belonging to the genus *Acinetobacter* and *Arthrobacter sp.* were responsible for PNP biodegradation. Consequently, in

this study the sum of *Acinetobacter* and *Arthrobacter sp.* was referred as the PNP-degraders fraction identified by FISH-CLSM.

Preparation and hybridization of samples were carried out according to the method developed by Suárez-Ojeda et al. (2011). Oligonucleotide probes were supplied by Thermo Scientific Biopolymers (Germany). The general probe UNIV1390 was used as positive control. Two specific probes were used being 3'-5' -end labelled ACA652 and 5' -end labelled KO-02, detailed information of the probes can be found on Table 4.1. Quantification of microbial population was done using the methodology described by Jubany et al., (2009).

Table 4.1. Tested probes targeting PNP-degraders (Suárez-Ojeda et al., 2011).

Probe Name	ProbeBase accession number	Specificity	Working solution (ng mL^{-1})	5' -end modification	Excitation/ Emission maximum (nm)	Molar extinction coefficient (M^{-1} cm^{-1})
UNIV1390	pB-00327	All bacteria	50	Cy5 (cyanine5)	649/670	250000
KO 02	pB-01256	*Arthrobacter sp.*	50	Cy3 (cyanine 3)	495/520	83000
ACA 652	pB-00012	Genus *Acinetobacter*	100	3'-5' -end Cy3 (cyanine 3)	550/570	150000

4.3. Results and discussion

4.3.1. SBR performance after transient PNP shock loads

Three experiments in the SBR-B receiving transient shock loads of PNP were carried out with the aim of assessing the resistance of the system to feed perturbations and the benefits of using bioaugmentation in a scenario of transient PNP shock loads.

First, a control experiment (i.e. without bioaugmentation) was carried out. A PNP shock load of 55 mg L^{-1} was introduced in the glucose-containing influent on day 1 as described in Figure 4.1a. The shock load was kept during 4 SBR-B cycles for a total period of 32 h.

The non-bioaugmented SBR-B exhibited poor efficiency in terms of PNP removal, achieving only 10% after the first cycle (Figure 4.2b). In the subsequent cycles after the shock load, PNP was accumulated in the reactor. Nevertheless, glucose biodegradation remained unaffected by the presence of PNP since the TOC concentration in the effluent corresponded completely to the non-removed PNP.

For the bioaugmentation experiments, two different dosages of PNP-degrading activated sludge were tried as bioaugmentation dosage may play a major role on improving the overall system performance (Chong et al., 1997). First, a fraction of PNP-degraders from SBR-A corresponding to the 2% w/w of the total biomass in SBR-B was added on day 1 (Figure 4.1b).

As can be seen in Figure 4.2d, bioaugmentation enhanced the PNP removal up to 84% within the first SBR-B cycle and reached up to 100% of PNP removal over the next SBR-B cycles. After 32 h of the PNP shock load, the influent was changed back to the wastewater containing only glucose as the sole carbon source. Glucose removal efficiency, measured as TOC, was the same than before the PNP shock load. SBR-B was normally operated (i.e. no PNP in the feeding) for a period corresponding to one SRT (20 days). After that, on day 21, another PNP shock load was performed to assess if the protecting activity of bioaugmentation still remained in SBR-B after one SRT. In the second shock load, PNP removal efficiency in the first SBR-B cycle was only 3% and increased slightly after 4 cycles (Figure 4.2d), probably due to activation of the PNP-degraders that still remained after one SRT without PNP in the feeding. This significant reduction in the efficiency comparing both shock loads, the one on day 1 and the other on day 21, may be a result of an intense competition or predation of the introduced microorganisms (PNP-degrading bacteria from SBR-A) by the indigenous bacteria of SBR-B (Van Limbergen et al., 1998), thus resulting in a washout of the PNP-degraders.

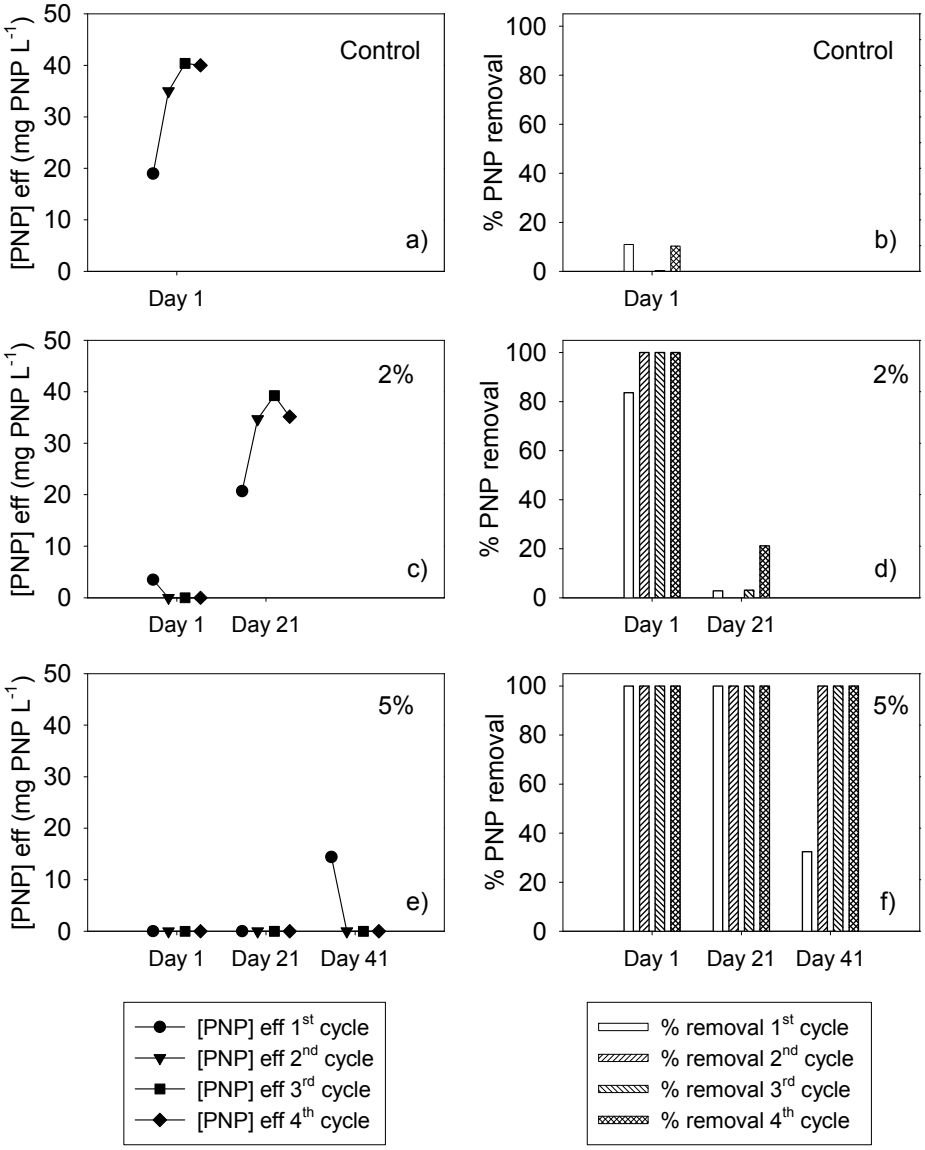


Figure 4.2. Transient PNP shock loads experiments: a) control experiment without bioaugmentation, b) bioaugmentation with 2% w/w of the total biomass in the seeded reactor and c) bioaugmentation with 5% w/w of the total biomass in the seeded reactor.

In the last experiment, a fraction of PNP-degraders from SBR-A corresponding to 5% w/w of the total biomass in SBR-B was added on day 1 (Figure 4.1c). From Figure 4.2f, one can see that PNP was completely removed in the first SBR-B cycle after the shock load. As in the previous experiment, after 32 h of PNP shock load, the influent was changed back to wastewater containing only glucose as the sole organic source. Glucose removal efficiency, measured as TOC, remained unaffected as before. Again, the SBR-B was normally operated for a period corresponding to one SRT (20

days) and then, the PNP shock load was repeated on day 21 and, in this case, PNP was completely removed in all the SBR-B cycles. This result was clearly better in terms of PNP removal efficiency than the achieved with a bioaugmentation dosage of 2% w/w. In this case, the PNP-degraders added to SBR-B could survive to the 20 days period without PNP.

In order to establish whether the 5% w/w dosage of PNP degraders from SBR-A could survive to two SRT, after 32 h of the PNP shock load on day 21, the influent was changed back to wastewater containing only glucose as the sole carbon source. Then, the PNP shock load was repeated on day 41, i.e. after two SRT. As can be seen on Figure 4.2f, in the first SBR-B cycle of day 41, the PNP removal efficiency was 32%, but it increased up to 100% over the next cycles. This result suggests that the PNP-degrading bacteria bioaugmented in SBR-B with a dosage of 5% w/w were able to survive to longer periods than the dosage of 2% w/w.

The intermediates from PNP aerobic biodegradation pathway, namely: 4-nitrocatechol, hydroquinone, catechol and 1,2,4-benzenetriol (Martín-Hernández et al., 2009; Spain and Gibson, 1991) were monitored in all the experiments but none of them were detected.

4.3.2. Assessing of PNP-degraders through FISH coupled with CLSM

The monitoring and quantification of the PNP-degraders was carried out in the three transient PNP shock loads experiments to validate the outcomes of the implemented bioaugmentation strategy. Sludge samples were taken from SBR-B just before every PNP shock load, i.e. on days 1, 21 and 41.

The PNP-degraders fraction was negligible in the activated sludge from a municipal WWTP used in the inoculation of SBR-B. This fact means that PNP-degrading activity found after the shock loads could be attributable to the specialized PNP-degrading bacteria added from SBR-A. For the experiment with a bioaugmentation dosage of 2% w/w, the PNP-degraders fraction decreased from 7 ± 5% on day 1 to 2 ± 2% after 20 days without PNP in the influent (Figure 4.3a), thereby confirming the partial washout of the added

PNP-degraders. The remaining fraction on day 21 was not able to remove completely the PNP of the second shock load but may explain the partial recovery of the PNP removal activity after 32 h of the second shock load (Figure 4.2d). For the experiment with a bioaugmentation dosage of 5% w/w, the PNP-degraders fraction went from 9 ± 6% on day 1 to 5 ± 3 and 4 ± 2% after 20 and 40 days without PNP in the influent, respectively (Figure 4.3b). However, in this experiment PNP shock loads were completely removed on days 21 and 41.

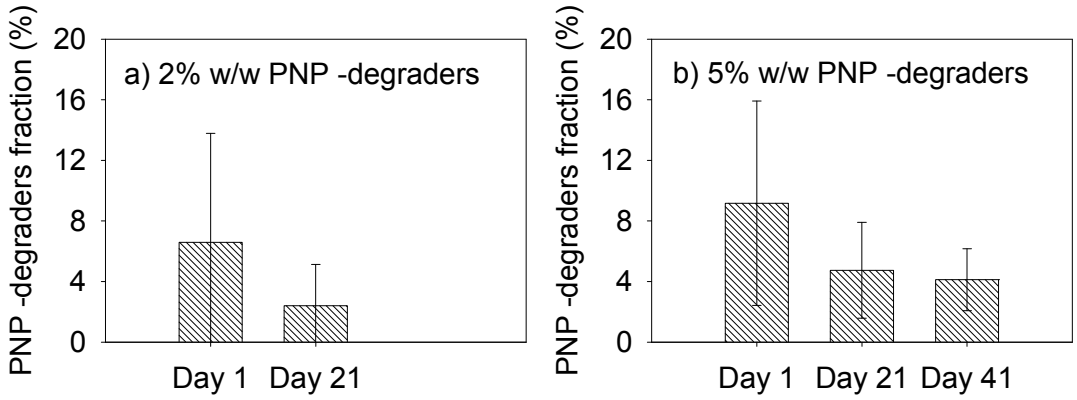


Figure 4.3. PNP-degrading bacteria fraction in the experiments with transient PNP shock loads: a) bioaugmentation with 2% w/w of the total biomass in the seeded reactor and b) bioaugmentation with 5% w/w of the total biomass in the seeded reactor.

Representative samples of the images obtained with CLSM for the bioaugmentation experiments can be seen in Figure 4.4 for the experiment with 5% w/w of PNP-degrading. In Figures 4.4b, 4.4e, and 4.4h, an image for the PNP-degrading bacteria on the experiment days 1, 21 and 41 is shown, Figures 4.4c, 4.4f, and 4.4i shows the merged image between the general probe and the specific probes for PNP-degrading bacteria, showing that it is a small fraction of the total bacteria and the loss of fluorescent signal that indicates a washout of the bacteria in the reactor. In Figure 4.5 the same trend can be observed for the experiments done with 2% w/w of PNP-degrading bacteria.

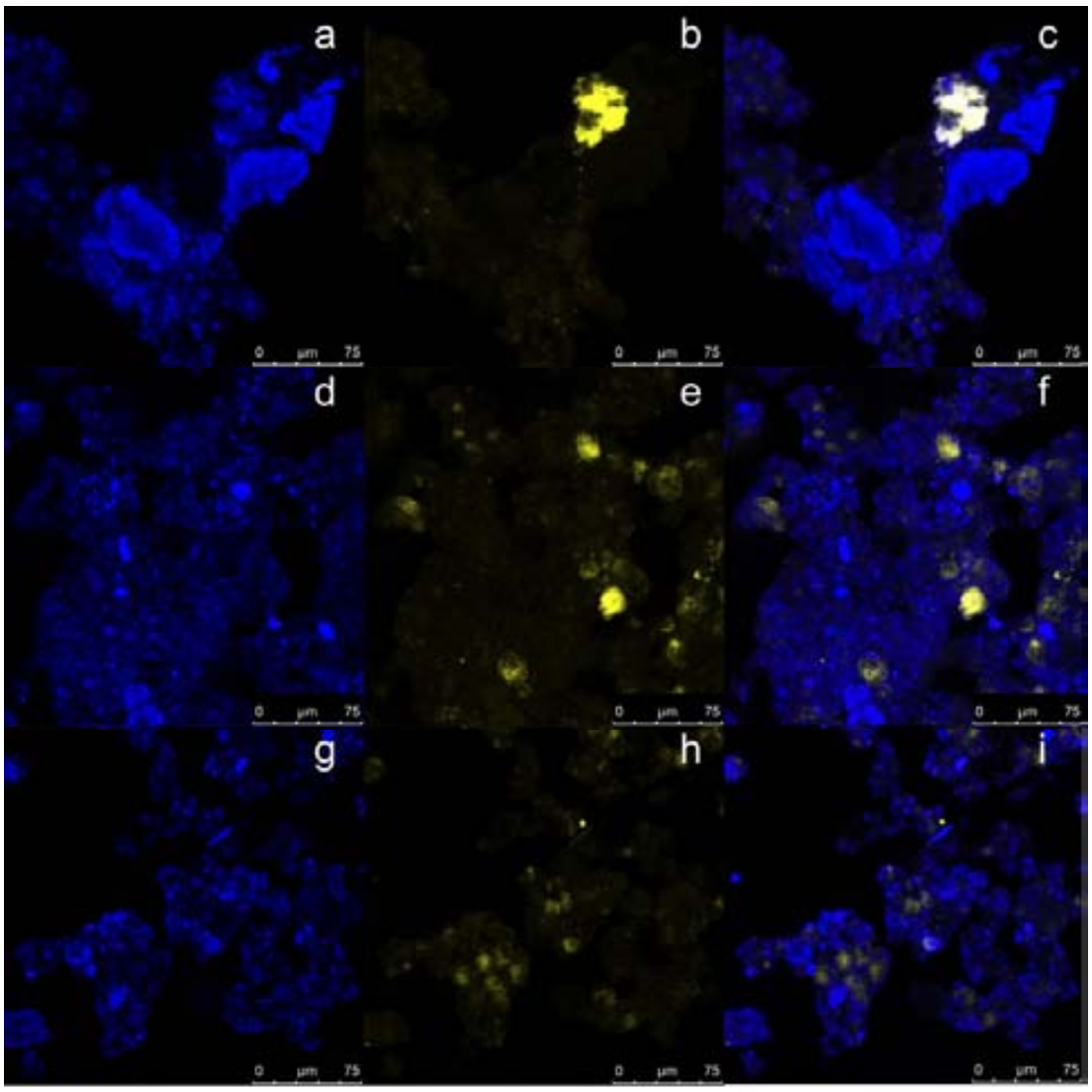


Figure 4.4. Samples of CLSM images obtained for bioaugmentation experiment using 5% w/w of PNP- degraders. Blue color: hybridized with Cy5-5'-end-labelled UNIV1390 probe (all bacteria). Yellow color: PNP-degraders probe hybridized simultaneously with Cy3-5'-end-labelled ACA652 probe (genus *Acinetobacter*) and CY3-5'-end-labelled KO 02 probe (*Arthrobacter* sp.). a) Day 1-General probe, b) Day 1-PNP degraders probe, c) Merged Image from a) and b). d) Day 20-General probe, e) Day 20-PNP degraders probe, f) Merged Image from d) and e). g) Day 40-General probe, h) Day 40-PNP degraders probe and i) Merged Image from g) and h). Scale bar = 75 µm.

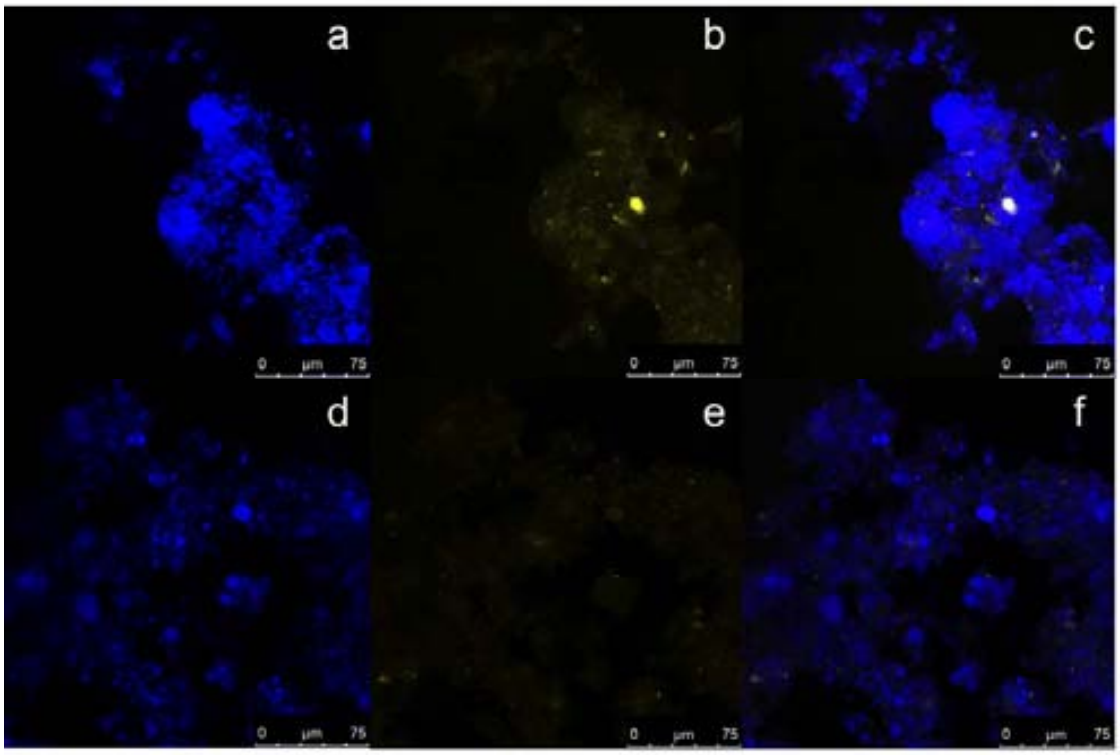


Figure 4.5. Samples of a CLSM images obtained for bioaugmentation experiment using 2% w/w of PNP- degraders. Blue color: hybridized with Cy5-5'-end-labelled UNIV1390 probe (all bacteria). Yellow color: PNP-degraders probe hybridized simultaneously with Cy3-5'-end-labelled ACA652 probe (genus *Acinetobacter*) and CY3-5'-end-labelled KO 02 probe (*Arthrobacter* sp.). a) Day 1-General probe, b) Day 1-PNP degraders probe, c) Merged Image from a) and b). d) Day 20-General probe, e) Day 20-PNP degraders probe, f) Merged Image from d) and e). Scale bar = 75 µm.

An explanation for the survival of the PNP-degrading bacteria after long periods without PNP could be the fact that glucose was used as co-substrate in the influent of both reactors, SBR-A and SBR-B. In this scenario, PNP-degraders may retain their microbial activity by using a readily biodegradable substrate (glucose) and therefore, being more resistant during a long starvation period. This effect is similar to the priming approach on soil remediation, where the population is predisposed to the future conditions it will encounter and thus obtaining a consortium of microorganisms possibly more resilient to stress and with more ability to survive (Singer et al., 2005).

Comparing both bioaugmentation experiments, it could be concluded that a higher initial dosage of specialized bacteria derives in a longer period of

survival of this biomass. Moreover, a minimum amount of PNP-degraders (around 4% for the operational conditions used in both experiments) could be necessary for a fast response against a transient shock load.

These results confirmed that the dosage of specialized bacteria is one of the most important operational parameters in bioaugmentation processes (Chong et al., 1997). Several studies that implemented successfully bioaugmentation strategies used larger dosages of specialized bacteria, around 25-32% w/w of the total biomass in the seeded reactor (Jianlong et al., 2002; Quan et al., 2004). From a practical point of view, the operation of a full-scale WWTP with such dosages would result in the need of another large-scale facility to develop the specialized bacteria. The results presented in this study indicate that the bioaugmentation with small dosages of specialized bacteria (around 2% w/w of the total biomass in the seeded reactor) may help overcome transient shock loads of a contaminant. Moreover, the use of slightly higher bioaugmentation dosages (around 5% w/w of the total biomass in the seeded reactor) may help to overcome future shock loads (up to two SRT).

4.3.3. SBR performance in front of a continuous PNP shock load

Two experiments, with and without bioaugmentation, in SBR-B receiving a continuous PNP shock load were carried out, with the aim of assess if bioaugmentation could decrease the start-up period needed to develop a SBR able to treat an influent containing PNP.

The first stage of the SBR-B operation in both experiments (15 days) corresponded to the inoculation with a municipal activated sludge (non-acclimated) using an influent containing only glucose as sole carbon source (data not shown). After this period, PNP was added in the feeding jointly with the glucose. Performance of both, control and bioaugmented reactor was monitored during 30 days. The operation conditions are summarized in Table 4.2.

On the one hand, for the control reactor (Figure 4.6a), on day 1 only 33%

of PNP removal was observed and gradually increased until 100% was reached on day 4. A general failure was observed after 8 days of operation in which the reactor could not handle the PNP loading rate resulting in PNP accumulation on the reactor. To overcome this problem, the feeding was interrupted (Figure 4.6b) and the reactor was kept under aeration until PNP was completely depleted and then, reactor operation was resumed. The first day after resuming the operation (day 11), PNP was still accumulated on the reactor but on the last days of operation, the reactor showed a slight improvement on its performance with an average of 91% of PNP removal.

On the other hand, in the bioaugmented reactor, the PNP-degrading bacteria were introduced on the SBR-B on day 1 and the influent PNP concentration was two-fold higher than the applied in the control reactor (Figure 4.6b). The PNP removal was almost complete from the beginning and thus, the volumetric PNP loading rate was increased progressively up to five-fold the applied in the control reactor (Figure 4.6c and Figure 4.6d) by reducing the HRT and finally by increasing the influent PNP concentration (Table 4.2).

Table 4.2. Operational conditions of the control and bioaugmented reactor under a continuous PNP shock load.

Operational conditions	Control reactor	Bioaugmented reactor			
Operational period (days)	1 - 30	1 - 8	9 -16	17 - 22	23 - 30
HRT (h)	18.4	18.4	14.1	9.4	9.4
PNP influent concentration (mg L^{-1})	38	82	82	82	273
PNP: glucose ratio (in terms of TOC)	6 : 94	10 : 90	10 : 90	10 : 90	32 : 68
Specific PNP loading rate (g PNP g^{-1} VSS d^{-1})	0.014	0.030	0.040	0.060	0.200

Comparing both experiments, the bioaugmented reactor showed a faster adaptation period to the continuous PNP shock load than the non-bioaugmented reactor. PNP accumulation episodes did not come up on the 30 days of operation of the bioaugmented reactor receiving a continuous PNP shock load. Moreover, the bioaugmented reactor showed a good resistance to successive increments of the applied volumetric PNP loading rate, while the

volumetric PNP loading rate could not be increased in the control reactor in 30 days. Similar results are reported by several studies (Ma et al., 2009; Wen et al., 2012).

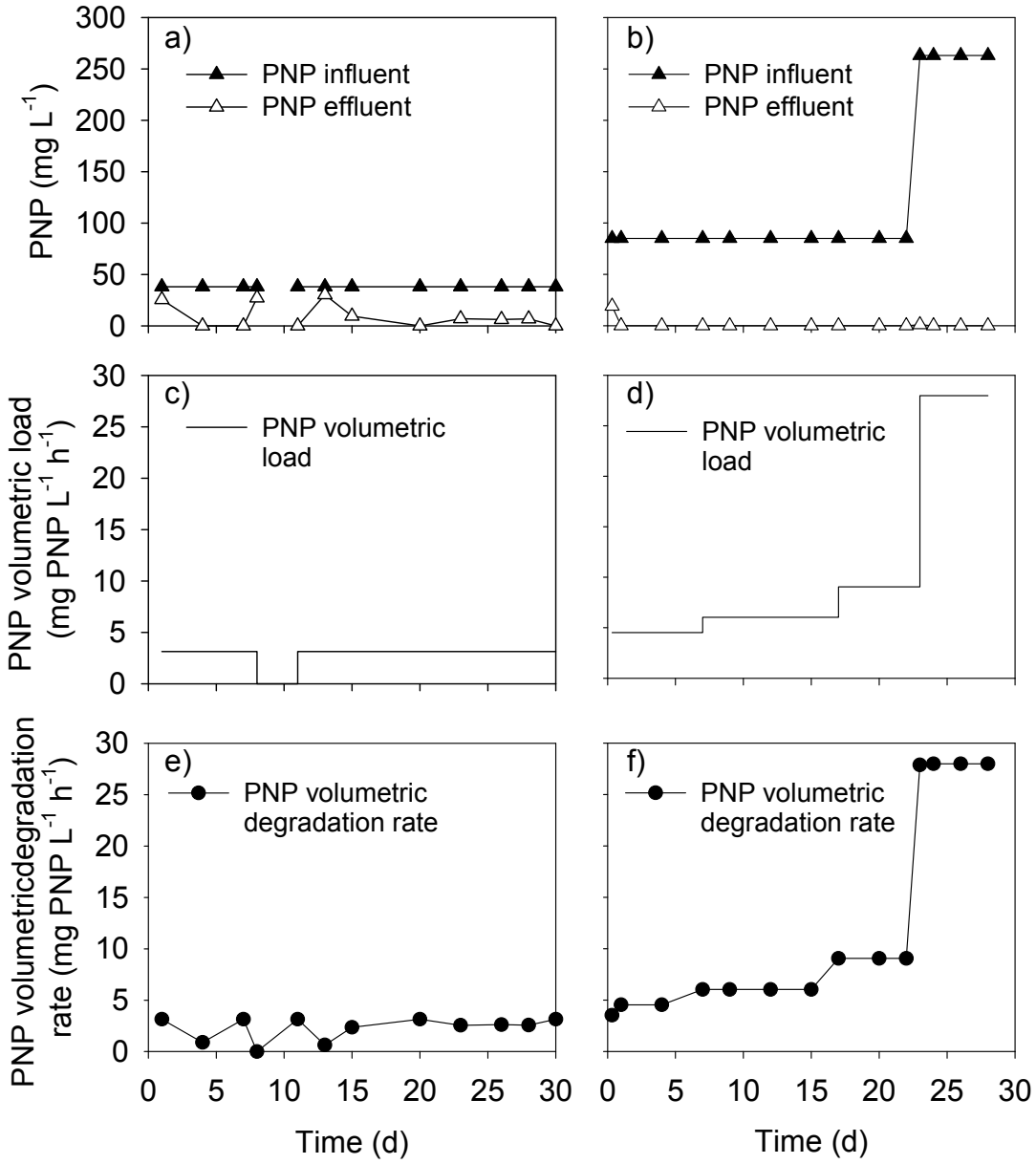


Figure 4.6. Continuous PNP shock load experiments. a), c) and e) control experiment without bioaugmentation; b), d) and f) bioaugmentation with 5% w/w of the total biomass in the seeded reactor.

Other important feature of the bioaugmentation strategy implemented in this study is that the addition of the specialized bacteria was done on a sole dosage at the beginning of the PNP shock load. In other studies,

bioaugmentation dosages had to be applied every day during the shock load period (Chong et al., 1997; Park et al., 2008; Schauer-Gimenez et al., 2010). The specific PNP loading rate reached in the bioaugmented reactor was 0.2 g PNP g^{-1} VSS d^{-1} that was about 14-fold higher than the initial one.

4.4. Conclusions

Bioaugmentation with PNP-degrading bacteria enhanced the removal of PNP in a SBR receiving transient or continuous PNP shock loads comparing to a non-bioaugmented SBR.

A successful bioaugmentation process can be achieved with small dosages of PNP-degrading bacteria (2-5% w/w of total biomass in the seeded reactor).

The key to the successful performance of a bioaugmented reactor under transient shock loads is the survival of the PNP-degraders after several SRT without PNP. In this sense, two factors may be critical: the dosage of PNP-degraders and the use of the same co-substrate in both the seeding and the seeded reactors.

4.5. References

APHA. 1998. *Standard methods for the examination of water and wastewater*. *20th ed ed*. American Public Health Association, NW Washington, DC. United States of America.

Bartroli, A., Carrera, J., Perez, J. 2011. Bioaugmentation as a tool for improving the start-up and stability of a pilot-scale partial nitrification biofilm airlift reactor. *Bioresource Technology*, 102(6), 4370-4375.

Chen, B.-Y., Chen, S.-Y., Lin, M.-Y., Chang, J.-S. 2006. Exploring bioaugmentation strategies for azo-dye decolorization using a mixed consortium of Pseudomonas luteola and Escherichia coli. *Process Biochemistry*, 41(7), 1574-1581.

Chong, N., Pai, S., Chen, C. 1997. Bioaugmentation of an activated sludge receiving pH shock loadings. *Bioresource Technology*, 59(2-3), 235-240.

El Fantroussi, S., Agathos, S.N. 2005. Is bioaugmentation a feasible strategy for pollutant removal and site remediation? *Current Opinion in Microbiology*, 8(3), 268-275.

Guo, J., Ma, F., Chang, C., Cui, D., Wang, L., Yang, J. 2009. Start-up of a two-stage bioaugmented anoxic-oxic (A/O) biofilm process treating petrochemical wastewater under different DO concentrations. *Bioresource Technology*, 100(14), 3483-3488.

Jianlong, W., Xiangchun, Q., Libo, W., Yi, Q., Hegemann, W. 2002. Bioaugmentation as a tool to enhance the removal of refractory compound in coke plant wastewater. *Process Biochemistry*, 38(5), 777-781.

Jubany, I., Lafuente, J., Carrera, J., Baeza, J. 2009. Automated thresholding method (ATM) for biomass fraction determination using FISH and confocal microscopy. *Journal of Chemical Technology and Biotechnology*, 84(8), 1140-1145.

Juteau, P., Larocque, R., Rho, D., LeDuy, A. 1999. Analysis of the relative abundance of different types of bacteria capable of toluene degradation in a compost biofilter. *Applied Microbiology and Biotechnology*, 52(6), 863-868.

Kulkarni, M., Chaudhari, A. 2007. Microbial remediation of nitro-aromatic compounds: An overview. *Journal of Environmental Management*, 85(2), 496-512.

Labana, S., Singh, O., Basu, A., Pandey, G., Jain, R. 2005. A microcosm study on bioremediation of p-nitrophenol-contaminated soil using Arthrobacter protophormiae RKJ100. *Applied Microbiology and Biotechnology*, 68(3), 417-424.

Ma, F., Guo, J., Zhao, L., Chang, C., Cui, D. 2009. Application of bioaugmentation to improve the activated sludge system into the contact oxidation system treating petrochemical wastewater. *Bioresource Technology*, 100(2), 597-602.

Martín-Hernández, M., Carrera, J., Pérez, J., Suárez-Ojeda, M. 2009. Enrichment of a K-strategist microbial population able to biodegrade p-nitrophenol in a sequencing batch reactor. *Water Research*, 43(15), 3871-3883.

Mohan, S., Rao, N., Prasad, K., Sarma, P. 2005. Bioaugmentation of an anaerobic sequencing batch biofilm reactor (AnSBBR) with immobilized sulphate reducing bacteria (SRB) for the treatment of sulphate bearing chemical wastewater. *Process Biochemistry*, 40(8), 2849-2857.

Mrozik, A., Piotrowska-Seget, Z. 2010. Bioaugmentation as a strategy for cleaning up of soils contaminated with aromatic compounds. *Microbiological Research*, 165(5), 363-375.

Nancharaiah, Y., Joshi, H., Hausner, M., Venugopalan, V. 2008. Bioaugmentation of aerobic microbial granules with Pseudomonas putida carrying TOL plasmid. *Chemosphere*, 71(1), 30-35.

Olaniran, A., Pillay, D., Pillay, B. 2006. Biostimulation and bioaugmentation enhances aerobic biodegradation of dichloroethenes. *Chemosphere*, 63(4), 600-608.

Park, D., Lee, D., Kim, Y., Park, J. 2008. Bioaugmentation of cyanide-degrading microorganisms in a full-scale cokes wastewater treatment facility. *Bioresource Technology*, 99(6), 2092-2096.

Plangklang, P., Reungsang, A. 2011. Bioaugmentation of carbofuran residues in soil by Burkholderia cepacia PCL3: A small-scale field study. *International Biodeterioration & Biodegradation*, 65(6), 902-905.

Quan, X., Shi, H., Liu, H., Wang, J., Qian, Y. 2004. Removal of 2,4-dichlorophenol in a conventional activated sludge system through bioaugmentation. *Process Biochemistry*, 39(11), 1701-1707.

Schauer-Gimenez, A., Zitomer, D., Maki, J., Struble, C. 2010. Bioaugmentation for improved recovery of anaerobic digesters after toxicant exposure. *Water Research*, 44(12), 3555-3564.

Singer, A., van der Gast, C., Thompson, I. 2005. Perspectives and vision for strain selection in bioaugmentation. *Trends in Biotechnology*, 23(2), 74-77.

Sipma, J., Osuna, M., Emanuelsson, M., Castro, P. 2010. Biotreatment of Industrial Wastewaters under Transient-State Conditions: Process Stability with Fluctuations of Organic Load, Substrates, Toxicants, and Environmental Parameters. *Critical Reviews in Environmental Science and Technology*, 40(2), 147-197.

Spain, J., Gibson, D. 1991. Pathway for biodegradation of para-nitrophenol in a Moraxella SP. *Applied and Environmental Microbiology*, 57(3), 812-819.

Suárez-Ojeda, M.E., Montón, H., Roldán, M., Martín-Hernández, M., Pérez, J., Carrera, J. 2011. Characterization of a p-nitrophenol-degrading mixed culture with an improved methodology of fluorescence in situ hybridization and confocal laser scanning microscopy. *Journal of Chemical Technology and Biotechnology*, 86(11), 1405-1412.

Van Limbergen, H., Top, E., Verstraete, W. 1998. Bioaugmentation in activated sludge: current features and future perspectives. *Applied Microbiology and Biotechnology*, 50(1), 16-23.

Vogel, T. 1996. Bioaugmentation as a soil bioremediation approach. *Current Opinion in Biotechnology*, 7(3), 311-316.

Wen, Q.X., Zhang, H.C., Chen, Z.Q., Zhao, Y., Feng, Y.J. 2012. Bioaugmentation for polyacrylamide degradation in a sequencing batch reactor and contact oxidation reactor. *Journal of Environmental Science and Health, Part A* 47(3), 358 - 365.

Yu, F.-B., Ali, S.W., Guan, L.-B., Li, S.-P., Zhou, S. 2010. Bioaugmentation of a sequencing batch reactor with Pseudomonas putida ONBA-17, and its impact on reactor bacterial communities. *Journal of Hazardous Materials*, 176(1-3), 20-26.

CHAPTER 5

Catalytic wet air oxidation of a high strength *p*-nitrophenol wastewater over Ru and Pt catalysts: Influence of the reaction conditions on biodegradability enhancement

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Catalytic and non-catalytic wet air oxidation experiments were done at the Institut de Recherches sur la Catalyse et l'Environnement de Lyon (IRCEL-YON), France during a research stay supervised by Dr. Claude Descorme.

Abstract

p-Nitrophenol (PNP) is widely used as a raw material in several industries, therefore it can be released to the environment, being mandatory the treatment of the PNP-contaminated industrial wastewaters. In this sense, the influence of temperature, oxygen partial pressure, type of catalyst, pH and ionic strength on the wet air oxidation (WAO) of a highly concentrated PNP wastewater was studied. Several 480 min batch tests have been performed and four Pt and Ru-based catalysts have been tested. The PNP elimination, total organic carbon (TOC) abatement and the intermediates distribution were monitored. Moreover, respirometric screening tests were completed after each experiment to assess the biodegradability enhancement of catalytic WAO (CWAO) treated effluents. The results showed that PNP elimination was higher than 90% in most cases, being the temperature the most important operating parameter upon CWAO. Additionally, all the catalysts showed a

similar behaviour in terms of PNP and TOC conversions. Besides, CWAO increased the biodegradability by more than 50% in most of the tested conditions, being the carboxylic acid fraction the key factor to be taken into account, as the best biodegradability enhancement was observed when this fraction was the highest. The partial pressure of oxygen had a negligible effect on the biodegradability enhancement. The ionic strength influence over the CWAO was studied and even though it did not affect the CWAO performances, the presence of NaCl in the solution resulted in a decrease of the effluent biodegradability. In terms of pH, the most suitable scenario was the one with no pH adjustment. Conclusively, this work demonstrated that an integrated CWAO and biological treatment would allow an easy removal of PNP and the intermediates formed during the first step of the treatment, being the best CWAO conditions for this pre-treatment to work at 180ºC under stoichiometric oxygen pressure (i.e. 7.6 bar of oxygen partial pressure) with a Ru/TiO_2 catalyst.

5.1. Introduction

Among all the possibilities for water pollution, industrial wastewaters represent a big challenge for researchers and technology developers. Intensive research is being made over removal of phenol and substituted phenols from wastewaters, not only because of their toxicity to the environment, but also, because of the large volumes disposed worldwide. For example in Europe more than 2000 ton yr^{-1} of phenolic compounds are released as indirect emissions to the environment (EPER, 2004). In this work, *p*-nitrophenol (PNP) has been selected as a model compound, because it is one of the phenolic compounds included in the list of High Volume Production Chemicals made by the Organization for Economic Cooperation and Development (OECD, 2008). PNP is primarily used in the N-acetyl-*p*-aminophenol production, which is a raw material in paracetamol production, as a chemical intermediate for the manufacture of insecticides (e.g. methyl and ethyl parathion), azo and sulphur dyes or leather preservatives (Bhatti et al., 2002; NLM, 1992). Besides its wide uses, PNP is highly toxic for the environment and human beings since repeated exposure may cause injury to

blood cells, damage to the nervous central system and have mutagenic effects; whereas long-term exposure could cause large damages to kidney and liver (Eichenbaum et al., 2009).

Biological treatment has been proved to degrade effectively PNP by using sequencing batch reactors (SBR) treating up to 400 mg PNP L^{-1}; however, the aerobic PNP degradation can be limited by substrate inhibition when high-strength concentrations of PNP wastewaters are treated (Carrera et al., 2011; Martín-Hernández et al., 2009; Tomei and Annesini, 2005).

In this sense, and among many others processes, Catalytic Wet Air Oxidation (CWAO) is an efficient technology which has been used to treat wastewaters containing organic compounds which are highly toxic or too concentrated to be treated only with biological treatment and it is used as one of the most economical and environmental-friendly oxidation process (Kim and Ihm, 2011). The process consists in oxidizing the pollutants under oxygen pressure at elevated temperature and in the presence of a catalyst, which can be metal oxides or noble metals, either heterogeneous or homogeneous (Oliviero et al., 2003a). The process can be directed in two different ways obtaining: (i) a complete mineralization of the organic pollutants into CO_2, N_2, H_2O and mineral salts or (ii) an increase of the effluent biodegradability by directing the conversion of the toxic organic compounds towards the formation of more biodegradable products, such as carboxylic acids (Di Iaconi et al., 2010). CWAO of substituted phenols has been widely studied using supported noble metals (Pd, Pt, Ru over ZrO_2, TiO_2, CeO_2, activated carbon and γ-Al_2O_3), CeO_2-supported Cu and Fe or bare activated carbons as catalysts, with temperature and pressure conditions ranging from 120 to 180ºC and 0.5 to 1.6 MPa of O_2 and focusing primarily on the performances of the catalyst, the pollutant conversion, the distribution of the partial oxidation products and more recently, on the biodegradability enhancement (Kim and Ihm, 2011).

Also, combinations of other oxidation processes, mainly Fenton and photo-Fenton, with biological treatment have been successfully studied for several industrial wastewaters (Di Iaconi et al., 2010). Moreover, ozonation of PNP has proven to enhance the biodegradability of the effluent (Adams et al., 1997). In addition, some of our previous works have demonstrated the

feasibility of using WAO and CWAO to increase the biodegradability of phenolic effluents (Suárez-Ojeda et al., 2007a; Suárez-Ojeda et al., 2007b).

In particular, for PNP oxidation, commercial activated carbons have been used as direct catalytic materials for PNP degradation in a trickle bed reactor, obtaining practically no conversion at 140ºC under 2 bar of O_2 partial pressure (Suárez-Ojeda et al., 2005). Other studies, using AC as catalyst as well, obtained nearly 80% of PNP conversion, but at the cost of increasing temperature and pressure (Santos et al., 2006). The same is observed when metal catalysts supported on metal oxides are used (Liou et al., 2010). The reaction intermediates were shown to be mainly hydroquinone, catechol, *p*-benzoquinone and carboxylic acids.

However, prior to discharge of the CWAO effluents into a conventional wastewater treatment plant (WWTP), the biodegradability and the toxic and inhibitory effects of these effluents have to be assessed to prevent any malfunctioning of the WWTP and to protect the biomass. To this end, several techniques and instruments can be used. BOD_5/COD ratio provides an approximate index of the proportion of the organic substances in the wastewater that are biodegradable under aerobic conditions; it is one of the most used techniques to determine the increase of biodegradability of the effluents (Karrer et al., 1997). Microtox® is also used successfully to determine the toxicity of an effluent, but it can result in an overestimation of the EC_{50} values (Ricco et al., 2004). The Zahn-Wellens test has also been used for toxicity screens, but it is time consuming and can underestimate this effect (Polo et al., 2011). Finally, respirometry uses the biomass directly taken from a WWTP. Therefore the effects of a determined influent to the WWTP are evaluated directly on the receiving sludge (Orupõld et al., 2001). Moreover, with respirometric tests, the influent organic matter can be classified as readily biodegradable, inert or toxic/inhibitory according to its effect on the receiving sludge (Suárez-Ojeda et al., 2007a).

Accordingly, the main objective of this work was to treat a high-strength PNP wastewater using CWAO and supported Ru and Pt catalysts, which have been used effectively for other substituted phenols (Li et al., 2007a). CWAO was performed as a pre-treatment to increase the biodegradability of the

influent before a subsequent biological treatment. To fix the most suitable conditions for a combined chemical and biological treatment of PNP contaminated wastewaters, the influence of temperature, O_2 partial pressure (P_{O_2}), pH, saline content and catalyst formulation on the PNP oxidation was assessed. Also, the biodegradability of these effluents was established through respirometry tests, providing valuable information to determine the most favourable conditions to perform a combined chemical and biological treatment on this type of wastewater. As far as the mineralization of PNP was not the pursued objective, but the optimization of the biodegradable fraction, the distribution of the reaction intermediates, which is directly related to the biodegradability enhancement, was also studied.

5.2. Materials and methods

5.2.1. Experimental set up for WAO and CWAO experiments

Experiments were performed in a 280 mL batch reactor made of Hastelloy. The reactor was equipped with a magnetically driven stirrer working at a constant velocity (1000 rpm), high enough to avoid external diffusion problems. Temperature was maintained constant in the range of 120 to 180ºC using an electric jacket; a schematic diagram for the reactor is given in Figure 5.1. For all the experiments, 140 mL of PNP solution at a given concentration and a certain amount of catalyst were introduced in the reactor. The solution was repeatedly purged with argon to remove any trace of oxygen and heated up to the desired reaction temperature under stirring. Once the temperature reached a steady state value, the stirrer was stopped and synthetic air (20 vol.% O_2 and 80 vol.% N_2) was introduced in the reactor up to the desired pressure. The stirrer was switched back on and this time was considered as the time zero for the reaction.

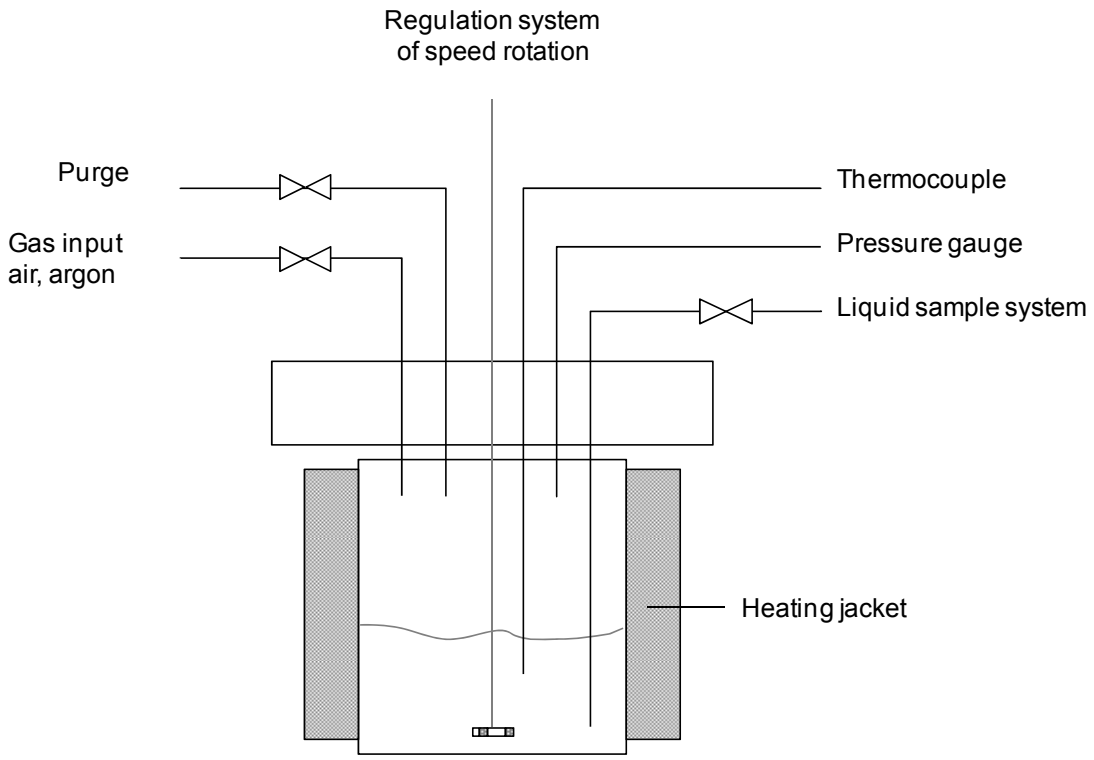

Figure 5.1. Batch reactor diagram for WAO and CWAO experiments.

Different operation conditions were evaluated with the objective to compare their influence on the intermediates distribution and the biodegradability enhancement (Table 5.1). All experiments were performed with an initial PNP concentration of 5 g L^{-1}, corresponding to an initial pH of 4.6 (without any pH adjustment, except wherever indicated in the text) and a stoichiometric oxygen partial pressure to achieve complete mineralization (except where indicated). All CWAO experiments were carried out using 0.5 g of a 3 wt.% metal catalyst (particle size: 20-40 μm), corresponding to a catalyst concentration of 3.6 $g_{catalyst}$ L^{-1}. The total pressure was also kept constant all along the experiment and hence re-adjusted whenever necessary, especially after each liquid sample withdrawal. The effect of temperature on the PNP treatment, both in the absence and in the presence of a catalyst, was tested at 120, 160 and 180ºC. The effect of the catalyst formulation was also tested using four different heterogeneous catalysts: Ru/TiO_2, Ru/ZrO_2, Pt/TiO_2 and Pt/ZrO_2. Other sets of experiments were performed to study the effect of the O_2 partial pressure (P_{O2}) at 160ºC and 180ºC. The initial pH effect was studied at three different values: 2.0, 4.6 and

8.0, adjusting the pH with H_2SO_4 or NaOH when needed. Finally, the impact of the solution ionic strength was studied by addition of a 25 g L^{-1} NaCl solution to the reaction mixture.

Liquid samples were periodically withdrawn from the reactor and further analyzed to follow the evolution of the PNP concentration and the formation of the reaction intermediates. Moreover, after the reaction, once the reactor reached ambient conditions, it was opened and its content was collected for further biodegradation analysis.

Table 5.1. Experimental conditions tested in the WAO and CWAO experiments.

AOP	Temperature (ºC)	Catalyst	P_{O_2} (bar)	O_2/PNP stoichiometric molar ratio	Initial pH	[NaCl] (g L^{-1})
WAO	120	-	7.6	1.0	4.6	0
	160	-	7.6	1.0	4.6	0
	180	-	7.6	1.0	4.6	0
CWAO	120	Ru/TiO_2	7.6	1.0	4.6	0
	160	Ru/TiO_2	7.6	1.0	4.6	0
	160	Ru/TiO_2	11.4	1.5	4.6	0
	180	Ru/TiO_2	7.6	1.0	4.6	0
	180	Ru/TiO_2	11.4	1.5	4.6	0
	180	Ru/TiO_2	7.6	1.0	2.0	0
	180	Ru/TiO_2	7.6	1.0	8.0	0
	180	Ru/ZrO_2	7.6	1.0	4.6	0
	180	Pt/TiO_2	7.6	1.0	4.6	0
	180	Pt/ZrO_2	7.6	1.0	4.6	0
	180	Ru/TiO_2	7.6	1.0	4.6	25

5.2.2. Catalyst preparation

Heterogeneous catalysts based on ruthenium (Ru) and platinum (Pt) were used in this study. Each metal was supported on two different metal oxides, namely TiO_2 and ZrO_2. Both supports are commercially available and their specifications are summarized in Table 5.2.

Table 5.2. Specifications of the commercial supports used in this work.

Support	TiO_2 DT51	ZrO_2 Mel
Supplier	Millennium	Melcat Chemical
Crystalline phase	Anatase	Monoclinic
Granulometry	Powder: 0.2-5 μm	Powder
S_{BET} (m^2 g^{-1})	80 – 100	90
Average pore diameter (nm)	9	8-9
Incipient volume (mL g^{-1})	1.1	0.5
pH_{pzc}	4.4	6.1

Ruthenium based catalysts were prepared via incipient wetness impregnation (Grosjean et al., 2010). The support was impregnated with a $Ru(NO)(NO_3)_3$ solution, using a volume corresponding to the incipient volume only. The metal loading was fixed at 3 wt.%. The solution was added to the support drop by drop under constant and vigourous stirring to guarantee an homogeneous dispersion of the metallic phase on the support.

Platinum based catalysts were prepared through the liquid impregnation method (Yang et al., 2010), using $Pt(NO_3)_2$ as the metal precursor. The Pt content was also fixed at 3 wt%. The mixture was stirred for 1 h at room temperature and then introduced in a rotary evaporator for 2 h at 60°C under reduced pressure (ca. 150 mbar).

The residual water was finaly eliminated by drying overnight in an oven set at 120°C. Both catalysts were reduced under flowing H_2 (15 L h^{-1}) at 300ºC for 2h. Finally, they were stored under argon (Béziat et al., 1999).

5.2.3. Analytical methods and materials

Several analyses were performed in order to determine the Total Organic Carbon (TOC) abatement, the PNP conversion and the reaction intermediates formation in each experiment.

PNP and all the others compounds used in this work were of analytical grade (Sigma–Aldrich, France). PNP and the reported intermediates in the PNP CWAO such as: 4-nitrocatechol (4-NC), phenol, 2,4-dinitrophenol (2,4-

DNP), biphenyl, *p*-benzoquinone (*p*-BQ), hydroquinone, catechol, succinic acid, maleic acid, fumaric acid, acetic acid, acrylic acid, formic acid, malic acid and glycolic acid were monitored (Castillejos-López et al., 2009; Santos et al., 2006; Suárez-Ojeda et al., 2007a). For the CWAO effluents, analyses were performed using High Performance Liquid Chromatography (HPLC) with a Shimadzu Prominence HPLC line equipped with a UV/Vis detector (UV SPD-20A). Compounds concentration was calculated by means of calibration curves established with external standards. For PNP and quinone-like compounds the column was a C18 QS Uptisphere 3 HDO (INTERCHROM, 100 x 2 mm, 3 μm). The flow rate was set at 0.3 mL min^{-1} and the UV/Vis detector at 254 nm. The mobile phase was a mixture of methanol and distilled water (2.5:97.5 v/v) acidified with H_3PO_4 (0.002N). For carboxylic acids, the column was a ICSepCoregel 107H Transgenomic (7.8 x 300 mm). In this case, the flow rate was set at 1.0 mL min^{-1} and UV/Vis detector was set at 210 nm. The mobile phase was distilled water acidified with H_2SO_4 (0.005N). In both cases, the injection volume was 20 μL and the column was maintained in an oven at 40°C. Samples were centrifuged at 60,000 rpm for 30 min prior to analysis. The standard deviation is 0.5%.

TOC and total nitrogen (TN) were measured using a Shimadzu TOC V_{CSH} analyzer, which was also provided with a TN analyzer module (TNM-1). The oven was set at 720ºC and the amount of CO_2 produced was measured by an infrared detector. The TN module was set at 50°C and used a chemilumiescent reaction to measure nitrogen species. The TOC and TN values were calculated by means of a calibration curve established with external standards. Two replicates of each sample were systematically measured and the average value was reported. The standard deviation was 4% in both cases.

Ionic chromatography analysis was used to measure nitrite and nitrate in the samples. The equipment used was an 881 ICpro Metrohm Compact, equipped with an auto sampler and two different modules for the analysis of anions and cations and the detection was performed by conductivity. The mobile phase was a solution of 3.2 mM of Na_2CO_3 and 1.0 mM of $NaHCO_3$. The column used was a METROSEP A Supp 5 150/4.0, the flow rate was set

at 0.7 mL min^{-1}, column temperature was set at 25°C and the injection volume was 20 µL.

pH analysis on the solution withdrawn from the reactor as a function of time were also performed with a PHM240 pH/ion Meter from Radiometer Analytical. The metal content on the catalyst was determined by ICP-OES.

5.2.4. Experimental set up for respirometric experiments and determination of biodegradation parameters

Respirometric tests allowed the determination of the readily biodegradable fraction of the Chemical Oxygen Demand (COD) of the final effluents collected at the end of each WAO and CWAO experiments. The experiments were performed in a LFS-type of respirometer, being the equipment and principle described elsewhere (Suárez-Ojeda et al., 2007b). Temperature and pH were set at 26 ± 0.5ºC and 7.5 ± 0.1, respectively.

The method for the determination of the biodegradation parameters was adapted from another study (Suárez-Ojeda et al., 2007b). For each respirometric test, biomass was taken from a full scale municipal WWTP located in the Barcelona area (Spain). Then, the sludge was aerated during two hours before experiment to remove any trace of soluble COD in the liquid. Three respirometric tests were performed to assess the biodegradability and the toxic or inhibitory effects of the selected effluents. The process might be summarized as follows: Run 1: 47 mg COD L^{-1} of sodium acetate, used as control substrate was added. Run 2: once the pulse in run 1 was consumed, a pulse of the WAO or CWAO effluent was added. Run 3: the pulse on Run 1 was repeated after pulse in Run 2 was completely depleted or after 1 h of contact time. The toxicity and inhibition effects can be assessed by comparing the Specific Oxygen Uptake Rate (SOUR) profiles and the oxygen consumed (OC) in runs 1 and 3 as follows:

$$\%\, Toxicity = \left(\frac{(OC_{RUN1})-(OC_{RUN3})}{(OC_{RUN1})}\right)\cdot 100 \qquad \text{Equation 5.1}$$

$$\%\, Inhibition = \left(\frac{(SOURmax_{RUN1})-(SOURmax_{RUN3})}{(SOURmax_{RUN1})}\right)\cdot 100 \qquad \text{Equation 5.2}$$

The biodegradability was measured as the percentage of readily biodegradable COD (COD_{RB}) according to the procedure described elsewhere by Suárez-Ojeda et al. (2007). COD_{RB} can be calculated from equations 5.3 and 5.4, using the heterotrophic yield coefficient (Y_H= 0.71 ± 0.2) obtained in a previous work (Suárez-Ojeda et al., 2007a) and the oxygen consumed (OC) measured during the respirometry:

$$COD_{RB} = \frac{OC}{1-Y_H}$$ Equation 5.3

$$\%COD_{RB} = \frac{COD_{RB}}{COD_{ADDED}} \cdot 100$$ Equation 5.4

COD_{ADDED} corresponds to 47 mg COD L^{-1} for all the effluents tested. Moreover, the biodegradability enhancement was considered as the difference between the $\%COD_{RB}$ in the influent and in the effluent after WAO or CWAO.

$$\text{Biodegradability enhancement} = (\%COD_{RB})_{eff} - (\%COD_{RB})_{inf}$$ Equation 5.5

It should be mentioned that the $\%COD_{RB}$ of a PNP sample of 47 mg COD L^{-1} was completely null. Consequently, the biodegradability enhancement after each experiment was directly the $\%COD_{RB}$ measured on the CWAO effluent.

5.3. Results and discussion

5.3.1. PNP degradation by WAO and CWAO

5.3.1.1. Effect of temperature on WAO and CWAO

The first step was to determine the thermal stability of the PNP molecule. This was evaluated at 180ºC in the absence of any catalyst and using 50 bar of argon instead of air. After 24 h, no PNP degradation was observed (data not shown). Consequently, any PNP degradation observed in the presence of air should result from oxidation and not from the thermal cracking of the PNP molecule.

To study the influence of temperature, WAO experiments in the absence of any catalyst were carried out at 120, 160 and 180ºC using the oxygen partial pressure corresponding to the stoichiometric amount required for complete mineralization. PNP and TOC conversions were significantly affected by temperature (Figure 5.2a and 5.2b). For instance, the PNP conversion after 480 min of reaction markedly increased with temperature, from a negligible value at 120ºC to 41 ± 1 and 90 ± 1% at 160 and 180ºC, respectively. The difference between the PNP and TOC conversions indicated the presence of partially oxidized compounds such as 4-NC, phenol, *p*-BQ, catechol and several carboxylic acids (succinic, fumaric and acetic acids). The increase in temperature also affected the reaction intermediates distribution, increasing the carboxylic acids fraction as temperature increased (see Figure 5.3). For the N-species, NO_2^- from PNP, 4-NC and 2,4-DNP molecules is oxidized to NO_3^-, which was the only N-compound detected. TN remained constant in all the experiments.

The effect of the use of a catalyst to improve the reaction rate and the final PNP conversion was assessed by comparing the WAO experiment performed at 180ºC with the CWAO experiment carried out under the same operational conditions but using Ru/TiO_2 as catalyst (Figure 5.2c and 5.2d). The use of the Ru/TiO_2 catalyst resulted in a low increase of the PNP conversion, but a higher TOC conversion (20 ± 4%), showing that the use of a catalyst acted on the intermediates distribution. The reaction is pushed further towards the formation of carboxylic acids at the end of the experiment (data not shown).

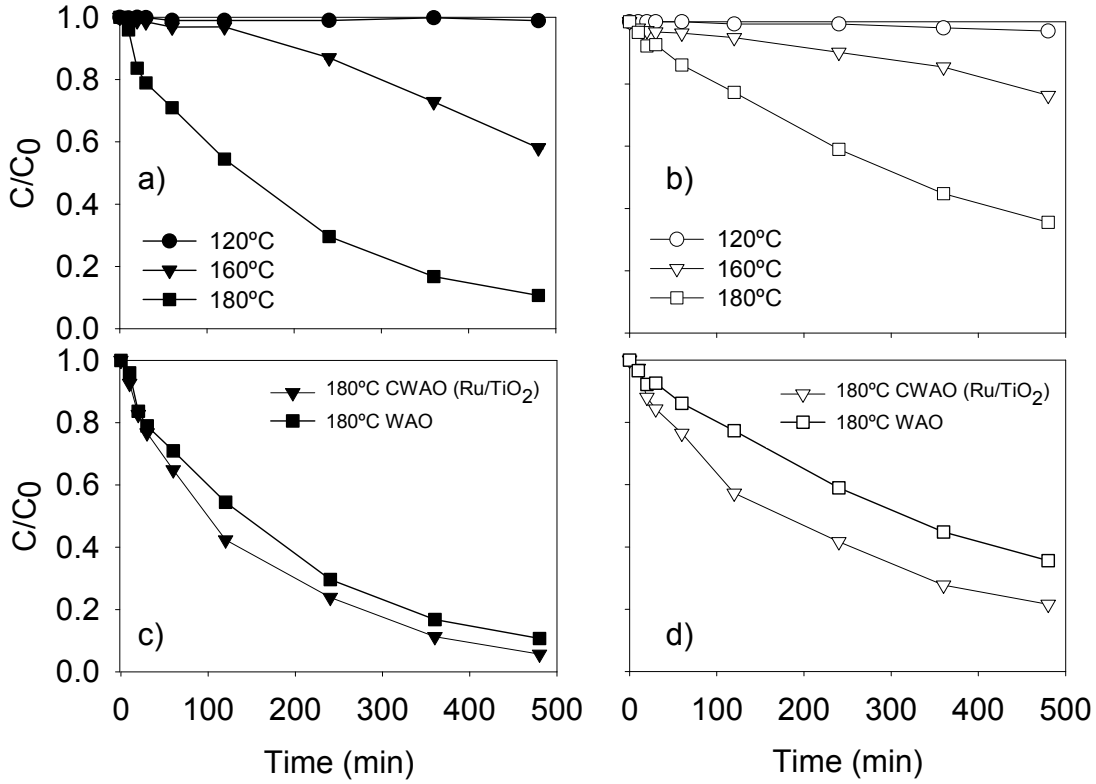

Figure 5.2 Evolution of the relative PNP and TOC concentrations as a function of time upon WAO and CWAO experiments under different reaction conditions. (a) PNP (full symbols) and (b) TOC (empty symbols) upon WAO at 120ºC – 7.6 bar of oxygen partial pressure (circles), 160ºC – 7.6 bar of oxygen partial pressure (triangles) and 180ºC – 7.6 bar of oxygen partial pressure (squares). (c) PNP (full symbols) and (d) TOC (empty symbols) upon WAO (squares) and CWAO (triangles) at 180ºC – 7.6 bar of oxygen partial pressure. Initial PNP concentration = 5 mg L^{-1}, 0.5 g of Ru/TiO_2. No pH adjustment. No NaCl.

In this case, the activation energy was directly derived from the Arrhenius plot (logarithm of the reaction rate versus the inverse of the temperature) and calculated to be ca. 57 ± 1 kJ mol^{-1} which is about half of the one calculated for the WAO experiments (101 ± 2 kJ mol^{-1}). Both values are comparable to the values reported in the literature for PNP oxidation (Atwater et al., 1997). Compared to the activation energy of the CWAO of 2-chlorophenol over Ru/ZrO_2 (36 kJ mol^{-1}) (Li et al., 2007b) and the CWAO of phenol over Pt-Ru/C (34 kJ mol^{-1}) (Atwater et al., 1997), PNP seems to be much more refractory to oxidation. These findings are in agreement with what has been reported by Pintar et al (1994), where the PNP degradation rate was shown to be slower than for phenol and 2-chlorophenol under the same oxidation conditions.

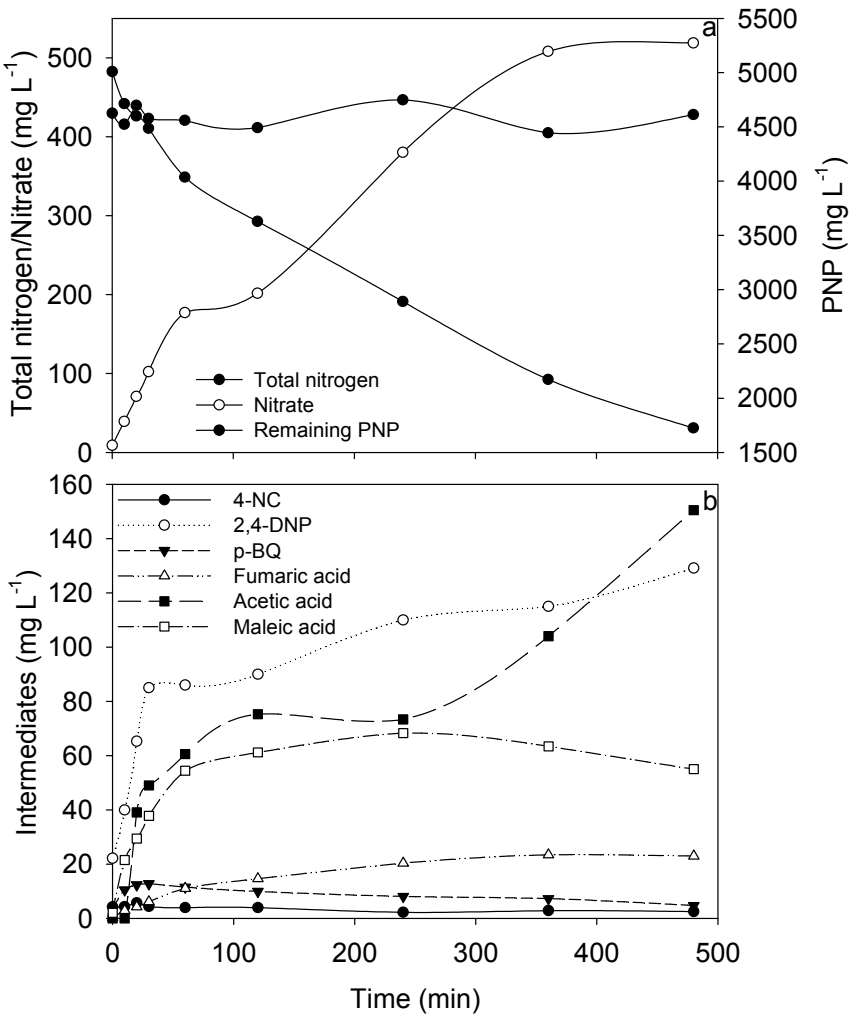

Figure 5.3. Example of time-course profiles for the intermediates distribution of a CWAO experiment at 160ºC and 11.4 bar of oxygen partial pressure. Initial PNP concentration: 5 g L^{-1}, 0.5 g of Ru/TiO_2, initial pH=4.6 and no NaCl.

Moreover, PNP contains a nitro group, which is an electron withdrawing substituent that strongly deactivates the benzene ring. This fact explains the high resistance to oxidation of PNP (Suárez-Ojeda et al., 2005). Moreover, it is well known that some carboxylic acids are refractory to CWAO. For example, the activation energy for the CWAO of succinic acid is 124 kJ mol^{-1} (Béziat et al., 1999). Consequently, it is expected that the reaction will not proceed further until complete mineralization under such reaction conditions. Nevertheless, the typical carboxylic acids present in the CWAO effluents from phenolic compounds (i.e. formic, succinic and acetic acid) were found to be readily biodegradable compounds, being suitable for a further biological treatment (Suárez-Ojeda et al., 2007b).

5.3.1.2. Effect of the catalyst formulation

As far as the use of a Ru/TiO_2 catalyst enhanced the fraction of carboxylic acids in the effluent, it was decided to test the activity of different Ru and Pt catalysts supported over TiO_2 and ZrO_2 to assess the possible effect of the type of catalyst on the PNP oxidation. The metal loading was fixed at ca. 3 wt.% for all catalysts and the operational conditions were: 180ºC, 7.6 bar P_{O2} and an initial PNP concentration of 5 g L^{-1}. To evidence any catalyst leaching, Ru, Pt, Zr and Ti concentrations were measured in the effluents: Ti and Zr concentrations were systematically below the ICP-OES detection limit (<0.1 mg L^{-1}), whereas the average leaching of Ru and Pt was 2.4 ± 0.2 and 1.5 ± 0.2% of the initial metal content, respectively. Ruthenium and platinum catalysts supported on Ce, Ti or Zr oxides are reported to be stable in the CWAO of organic compounds with low concentration of metal in the effluents or concentrations even below the detection limit (Li et al., 2008; Yang et al., 2010). Nevertheless, it is also reported in the literature that some nitrogen species, with a single pair of electrons on the nitrogen atom, can create a strong chemical bond with the metal atoms (Grosjean et al., 2010). However, none of these species were detected in the effluents for the tested catalysts.

The initial reaction rate was of the same order of magnitude for Ru/TiO_2, Ru/ZrO_2, Pt/TiO_2 and Pt/ZrO_2, i.e. 18, 20, 22 and 22 mol_{PNP} mol^{-1} h^{-1}, respectively; whereas the final PNP conversion after 480 min reaction was of 94 ± 1, 97 ± 1, 98 ± 1 and 98 ± 1%, respectively (Figure 5.4a). Looking at the TOC conversion after 480 min reaction, the values obtained were 78 ± 4, 81 ± 4, 81 ± 4 and 80 ± 4%, respectively (Figure 5.4b). These results showed that there is no significant difference between the PNP and TOC conversions, nor in the intermediates distribution, despite of the use of different catalysts. However, in other studies, Ru was found to be more active than Pt catalysts for carboxylic acids (Barbier-Jr et al., 1998), phenol and aniline (Barbier-Jr et al., 2005), whereas the opposite trend was observed in the ammonium CWAO (Barbier-Jr et al., 2002).

In any case, considering that Ru and TiO_2 are cheaper than Pt and ZrO_2 and because of the negligible differences between catalysts, the rest of the study was carried out using the Ru/TiO_2 catalyst.

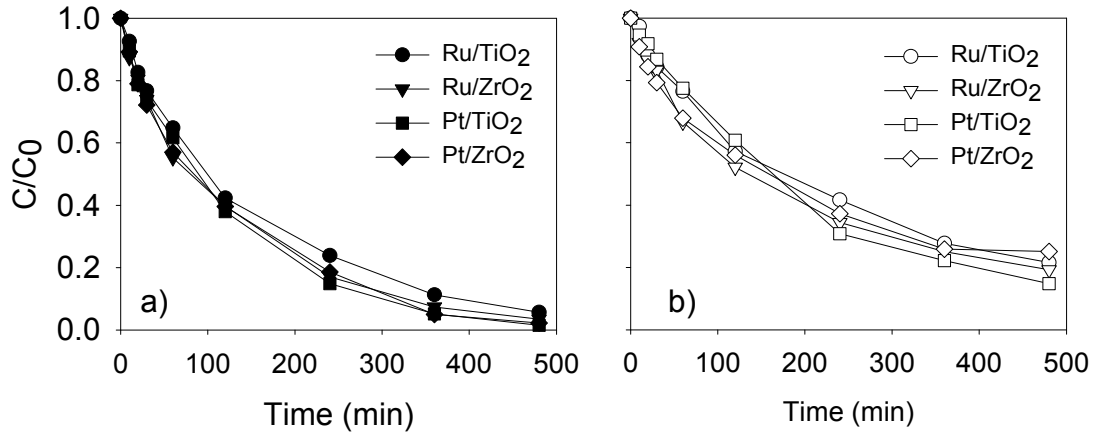

Figure 5.4. Evolution of the relative PNP and TOC concentrations as a function of time upon CWAO experiments using different catalyst. (a) PNP (full symbols) and (b) TOC (empty symbols) over Ru/TiO_2 (circles), over Ru/ZrO_2 (triangles), over Pt/TiO_2 (squares) and over Pt/ZrO_2 (diamonds) at 180ºC – 7.6 bar of oxygen partial pressure. 0.5 g of catalyst. No pH adjustment. No NaCl.

5.3.1.3. Effect of initial pH

The effect of pH over the PNP oxidation was assessed at three different pH values: 2.0, 4.6 (without any pH adjustment) and 8.0. The pH was adjusted at the beginning of the reaction using either sodium hydroxide or sulphuric acid to set the initial pH at 8.0 and 2.0, respectively. The reaction was performed at 180ºC under 7.6 bar oxygen partial pressure and with an initial PNP concentration of 5 g L^{-1} over a 3 wt.% Ru/TiO_2 catalyst. The pH profiles for each experiment, as well as the intermediates evolution, can be seen on Figure 5.5.

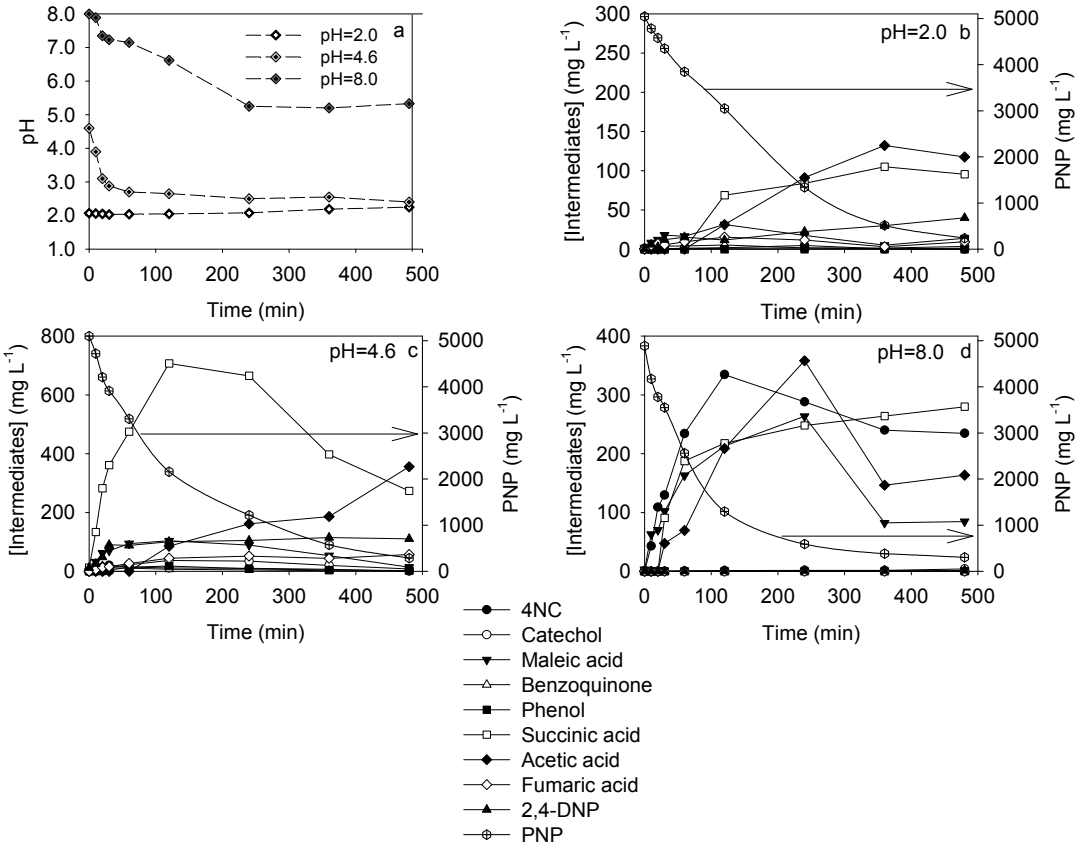

Figure 5.5. Time-course profiles for pH influence (a) and intermediates generated at pH 2.0 (b), pH 4.6 (c) and pH 8.0 (d). CWAO performed with an initial PNP concentration of 5 g L^{-1}, 0.5 g of Ru/TiO_2, at 180ºC and 7.6 bar of oxygen partial pressure.

It was found that the higher the initial pH, the higher the initial reaction rate: 9.0 ± 0.1 at pH=2.0, 17.0 ± 0.1 at pH=4.6 and 22.0 ± 0.1 mol_{PNP} mol_{Ru}^{-1} h^{-1} at pH=8.0. Nevertheless, at the end of the experiment (480 min) neither the PNP nor the TOC conversions (94 ± 1% and 78 ± 4%, respectively) were affected by the initial pH value. These results were different from the pH effect reported by Pintar et al. in the CWAO of substituted phenols (Pintar and Levec, 1994), when at pH higher than the pKa, the phenolate anion was more reactive than the protonated form. Similar trends were also observed over activated carbon catalysts (Santos et al., 2006), where higher PNP conversions were achieved at basic pH. Actually, under acidic conditions, the concentration of toxic reaction intermediates was reported to be higher than under basic conditions. On the contrary, the CWAO of PNP over a Fe(III) resin (Liou et al., 2010) was favoured at pH below the pKa of PNP with 100%

PNP conversion, but a lower degradation rate. The same happened upon sonophotocatalytic (Mishra and Gogate, 2011) and wet electrocatalytic oxidations (Dai et al., 2008).

In spite of the same PNP and TOC conversions, there were several differences in the intermediate distribution. Looking at the products in the effluent when the initial pH was 2.0, a larger fraction of the reaction intermediates remained unidentified (see Figure 5.9d below). At pH 8.0, there was a higher fraction of non-oxidized phenolic compounds. Moreover, the biodegradable acids fraction was higher when the initial pH was 4.6. These differences could be attributed to different reaction routes, as previously reported in (Santos et al., 2006).

5.3.1.4. Effect of oxygen partial pressure

The effect of the oxygen partial pressure was studied at two different temperatures: 160 and 180 ºC, using two different oxygen partial pressures related to the stoichiometric amount of oxygen required for complete mineralization of PNP and a 50% oxygen excess compared to the stoichiometric amount. Noteworthy, the total pressure was also kept constant all along the experiment and so re-adjusted whenever necessary, especially after each liquid sample withdrawal. All the other reaction conditions were kept constant: 3 wt.% Ru/TiO_2 catalyst, initial pH of 4.6 and initial PNP concentration of 5 g L^{-1}. The initial reaction rate remained almost constant for all the experiments performed at a given temperature, i.e. ca. 8 ± 1 and 18 ± 1 mol_{PNP} mol_{Ru}^{-1} h^{-1} at 160 and 180ºC, respectively. These values have been calculated from the data appearing in Figure 5.6a and Figure 5.6b. The results demonstrate that oxygen transfer from the gas to the liquid phase and from the liquid phase to the catalyst surface was not rate limiting (Bo et al., 2006).

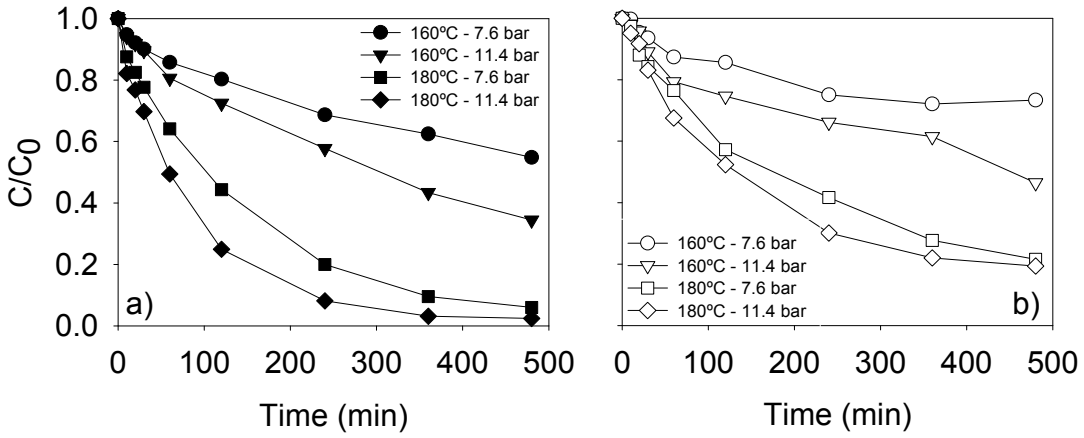

Figure 5.6. Evolution of the relative PNP and TOC concentrations as a function of time upon CWAO experiments under different reaction conditions (a) PNP (full symbols) and (b) TOC (empty symbols) upon CWAO at 160ºC - 7.6 bar (circles), 160ºC - 11.4 bar (triangles), 180ºC - 7.6 bar (squares) and 180ºC - 11.4 bar (diamonds). Initial PNP concentration = 5 mg L^{-1}, 0.5 g of Ru/TiO_2. No pH adjustment. No NaCl.

5.3.1.5. Effect of ionic strength

The effect of the ionic strength of the influent on the PNP oxidation and the biodegradability enhancement was studied with two experiments carried out under the same operational conditions (180ºC, 7.6 bar P_{O2}, initial PNP concentration of 5 g L^{-1} 3 wt.% Ru/TiO_2 catalyst) but using two different NaCl concentrations (0 and 25 g L^{-1}). Upon addition of sodium chloride, the initial reaction rate decreased from 18 to 13 mol_{PNP} mol_{Ru}^{-1} h^{-1}. However, at the end of the experiment (480 min), the PNP and TOC conversions and the intermediates distribution were similar in both experiments. Similarly, Béziat et al reported that inorganic salts, such as sodium chloride, slightly decreased the oxidation rate of acetic acid upon the CWAO of succinic acid (Béziat et al., 1999). In any case, this result is very encouraging since most of the industrial effluents demonstrate high ionic strengths, as simulated here by the addition of a large amount of NaCl.

5.3.2. Intermediates distribution and reaction pathway

The reaction intermediates detected in this study were catechol, phenol, 4-NC, 2,4-DNP, *p*-BQ and fumaric, acetic, succinic, maleic and acrylic acids. Depending on the operational conditions, some of these intermediates were present in higher, lower or even null concentrations. The concentration of each intermediate and the unconverted PNP at the end of each experiment (480 min) are presented in Figure 5.7. Regarding N-species, as we said before, the only intermediate detected was NO_3^-, being the TN constant, as expected. It should be mentioned that, the biological treatment proposed in this study for being after the CWAO is based on aerobic heterotrophic microorganisms, so that the N-species were not further studied.

Intermediates were only present in minor amounts after the WAO and CWAO experiments at 120ºC since the PNP conversion was very limited even after 480 min (Figure 5.7a and Figure 5.7b). In general, the concentration of carboxylic acids (mainly acetic and succinic) increased with increasing temperature in both WAO and CWAO experiments (Figure 5.7a and Figure 5.7b). Moreover, the use of a catalyst prevented the accumulation of 4-NC, especially at 160ºC (Figure 5.7a and Figure 5.7b). The nature of the catalysts had almost no effect on the intermediates distribution (Figure 5.7c), being the only remarkable difference the accumulation of fumaric acid with the Ru-based catalysts. The increase of oxygen concentration had only a limited effect on the intermediates distribution whatever the temperature (Figure 5.7d). The accumulation of 2,4-DNP increased with increasing the oxygen partial pressure, whereas maleic acid only appeared under stoichiometric oxygen partial pressure. Finally, the initial pH of the reaction mixture had a significant impact on the intermediates distribution (Figure 5.7e). At basic pH (8.0), a high concentration of 4-NC accumulated in the reactor, while it was almost zero at acid pH values (2.0 and 4.6). The highest accumulation of carboxylic acids happened at pH 4.6, with mainly acetic and succinic acids. The lowest carboxylic acids accumulation happened at the lowest pH (2.0), when the highest concentration of 2,4-DNP was detected.

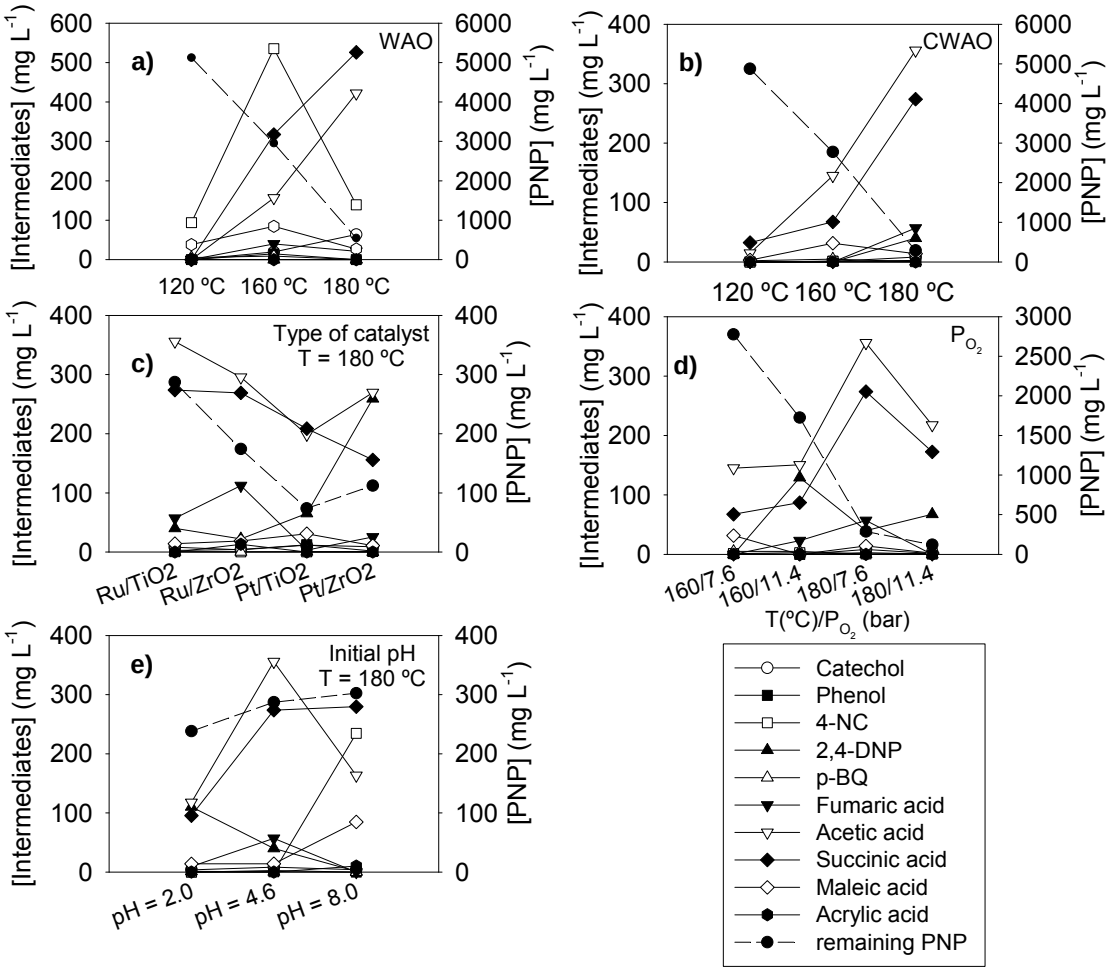

Figure 5.7. Intermediates distribution at the end of the reaction upon different experiments (see Table 1): (a) WAO experiments at different temperatures, (b) CWAO experiments at different temperatures, (c) impact of the catalyst formulation, (d) influence of oxygen partial pressure and (e) impact of the initial pH.

The identification of all by-products and intermediates formed upon reaction is critical to evaluate the toxicity and the biodegradability of CWAO effluents, as far as the residual TOC value does not provide enough information to assess these parameters. However, this is a hard task since, in the case of nitro-aromatics, the possible intermediates are quite diverse and complex and sometimes present at very low concentration, so that a proper identification and quantification becomes difficult. For that reason, the information available in the literature about the nitro-aromatics intermediates formed upon CWAO is limited (Oliviero et al., 2003a). It is generally accepted

that the CWAO of phenol and aniline proceeds via a free radical mechanism (Castillejos-López et al., 2009; Collado et al., 2011; Gomes et al., 2008; Liotta et al., 2009; Oliviero et al., 2003a; Oliviero et al., 2003b; Yang et al., 2008). The reaction pathway proposed for the CWAO of PNP is schematized on Figure 5.8, in line with what was established by Pintar and Levec (1994) in the CWAO of substituted phenols and aniline. In that study, PNP was indeed found to be an intermediate in the aniline CWAO and the results observed in our study confirmed that the reaction pathway might follow the same free radical mechanism, as the one observed in the CWAO of phenol and aniline.

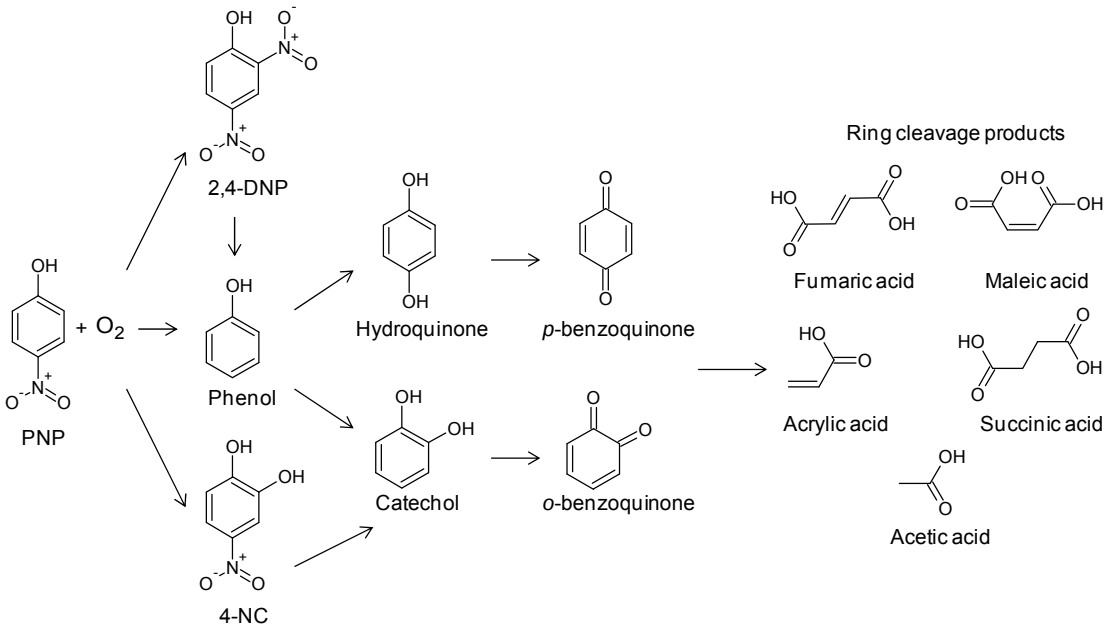

Figure 5.8. Proposed reaction pathway for the CWAO of PNP.

In the proposed pathway, the first step would be the cleavage of the N-C bond to give a phenoxy radical. Then, the formation of 2,4-DNP and 4-NC could be explained by the presence of the nitro group, which has an electron withdrawing effect in the *para* position of the aromatic ring, reducing partially the effect of the hydroxyl group, and therefore, reducing the rate of the electrophilic attack and the subsequent formation of catechol and hydroquinone. This effect was also reported by Collado et al. (2011) but using *p*-hydroxybenzoic acid, which also presents the same electron withdrawing effect. Thus, by a resonance effect of the PNP molecule, the nitro group would be a *meta* directing group since the substitution on the *ortho and para*

positions would be less stable. This could be the reason why, under the tested conditions, the formation of phenoxy radicals was hindered by steric effect of the nitro group. As a result, 4-NC was formed by the electrophilic attack of the PNP molecule at the early stages of the oxidation. Later on, the rupture of the N-C bond in 4-NC led to the formation of catechol, as shown on Figure 5.8. After catechol and hydroquinone were formed, a subsequent proton transfer led to *p*-BQ and the unstable *o*-benzoquinone. Later the C-C bond breaking led to ring opening products, mainly carboxylic acids. As it is said in section 5.3.1.1, the presence of NO_3^-, can be explained by the oxidation of NO_2^-, which comes from the PNP, 4-NC and 2,4-DNP molecules. As expected the total nitrogen remained constant.

Other oxidation processes, like electrochemical, electrocatalytic and microwave assisted oxidations also followed a free radical mechanism through phenoxy radicals as well, but with different termination steps and resulting in different intermediates (Bo et al., 2006; Dai et al., 2008; Jiang et al., 2010; Ogunbayo et al., 2011; Quintanilla et al., 2010; Zhu et al., 2010).

5.3.3. PNP CWAO effluents and intermediates biodegradability

In a previous study, a new methodology based on respirometry was established to classify the reaction intermediates formed upon CWAO into readily biodegradable, inert and toxic/inhibitory compounds, according to their effect on the Specific Oxygen Uptake Rate (SOUR) and the Oxygen Consumption (OC) (Suárez-Ojeda et al., 2007b). In this former study, the reaction intermediates presenting a similar OC (for the same initial COD concentration) to a readily biodegradable control substrate (acetic acid) were considered as readily biodegradable intermediates. When the compounds were not readily biodegradable, but did not have any toxic or inhibitory effect over the consumption of the control substrate, they were classified as inert intermediates at the tested concentrations. These inert intermediates could be slowly biodegraded but, to confirm it, a different and high-time consuming experiment would have been necessary. Finally, when the intermediates showed a negative effect over the consumption of the control substrate, they were classified as toxic or inhibitory for a further biological treatment.

Following the same methodology, some of the reaction intermediates identified in the present study could be separated according to this classification.

However, PNP and other intermediates such as: 4-NC, acrylic acid and 2,4-DNP had not previously been tested and subsequently classified. Therefore, using the respirometric procedure previously described (section 5.2.4), PNP was classified as an inert compound since its COD_{RB} was just 2.2 ± 0.7%, exhibiting a slight inhibitory effect and only a moderate toxic effect at 47 mg COD L^{-1}.

For 4NC and 2,4-DNP, the toxic and inhibitory effects were less than 5% at 47 mg COD L^{-1}. Consequently, both compounds were classified as inert intermediates. Finally, acrylic acid effect was studied at different concentrations between 26 and 107 mg COD L^{-1}. The $\%COD_{RB}$ was around 20%, which could allow its classification as a biodegradable intermediate. However, at high concentration, acrylic acid also exhibited a marked inhibiting effect (up to 80% at 107 mg COD L^{-1}) and consequently, it was classified as an inhibitory intermediate.

According to our previous results (Suárez-Ojeda et al., 2007b) and the respirometric tests carried out in this study, the intermediates of the PNP oxidation were classified as: i) readily biodegradable compounds: acetic, fumaric, and succinic acids; ii) inert compounds: PNP, phenol, catechol, 4-NC, 2,4-DNP and maleic acid; iii) toxic/inhibitory compounds: p-BQ and acrylic acid. For obvious reasons, the individual effect of the unidentified compounds could not be determined.

In order to establish the optimal conditions for a combined chemical-biological treatment, respirometric tests were performed on all the CWAO effluents described in section 5.3.1. The objective was to find the best operational conditions in terms of biodegradability enhancement that would allow a successful coupling with a subsequent biological treatment. The readily biodegradable COD fraction (COD_{RB}) was calculated using the procedure detailed in section 5.2.4.

Figure 5.9 shows the $\%COD_{RB}$ of each effluent together with the

corresponding intermediate distribution, according to the classification obtained from the respirometric tests. Both effluents obtained upon WAO (Figure 5.9a) and CWAO (Figure 5.9b) at 120ºC exhibited a negligible $\%COD_{RB}$. The effect of increasing the temperature was remarkably higher on the $\%COD_{RB}$, i.e. in terms of biodegradability, than on the TOC and PNP conversions. For instance, the $\%COD_{RB}$ upon CWAO with a Ru/TiO_2 catalyst (Figure 5.9b) increased from 4% at 120ºC to 30% at 160ºC and 61% at 180ºC. These results are in agreement with what was found in the CWAO of phenol and *o*-cresol by Suárez-Ojeda et al. (2007a), when it was demonstrated that an increase in temperature and the use of a catalyst improved the biodegradability, because of the higher concentration of readily biodegradable intermediates. However, the $\%COD_{RB}$ achieved in this study using noble metal catalysts was higher than the one reported by Suarez-Ojeda et al. using activated carbon (AC) as catalyst (Suárez-Ojeda et al., 2007a). Consequently, the use of noble metal catalysts improved the biodegradability of the CWAO effluents compared to other catalysts such as AC. The results obtained upon WAO and CWAO at 160 and 180ºC were clearly different in terms of biodegradability, despite the small increase of temperature (Figure 5.9a and Figure 5.9b). The reason is the extraordinary increase of the biodegradable intermediates fraction obtained with the increase of temperature. Moreover, the $\%COD_{RB}$ values (55-60%) achieved at 180ºC after WAO or CWAO were very similar or even better than those obtained for urban wastewaters. Consequently, these effluents could clearly be considered as biodegradable.

The use of different catalysts had only a slight influence on the biodegradability enhancement since the $\%COD_{RB}$ obtained with the four different catalysts was very similar: 61% for Ru/TiO_2, 56% for Ru/ZrO_2, 64% for Pt/TiO_2 and 60% for Pt/ZrO_2. However, there seems to be a slight relationship between the support and the biodegradability enhancement, being the catalysts supported on TiO_2 the one leading to the effluents with the highest biodegradability.

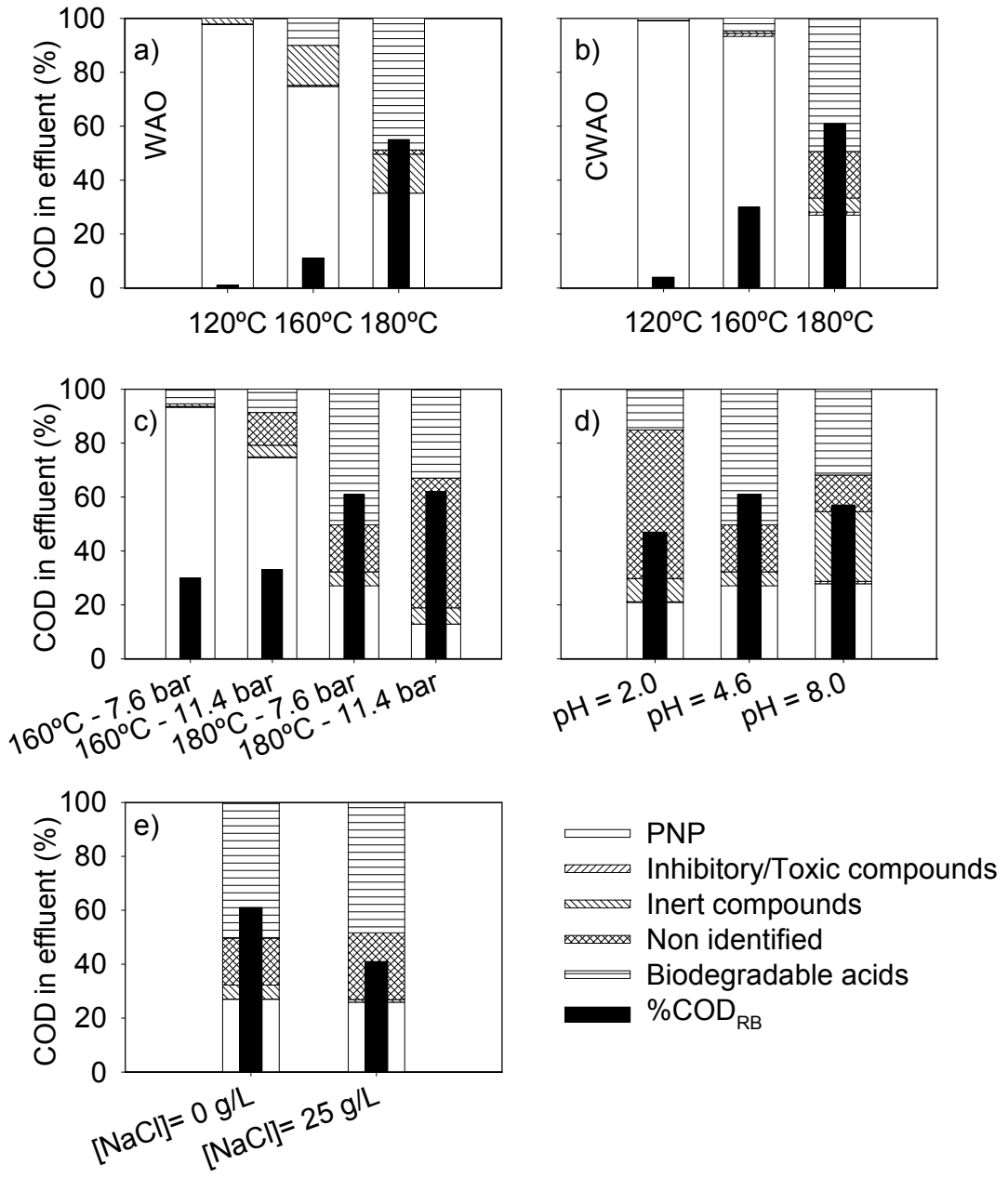

Figure 5.9. Intermediates distribution in terms of biodegradability and %COD_{RB} in the WAO and CWAO effluents: (a) WAO experiments at 120ºC – 7.6 bar of oxygen partial pressure; 160ºC – 7.6 bar of oxygen partial pressure; 180ºC – 7.6 bar of oxygen partial pressure; (b) CWAO over Ru/TiO_2 at 120ºC – 7.6 bar of oxygen partial pressure; 160ºC – 7.6 bar of oxygen partial pressure; 180ºC – 7.6 bar of oxygen partial pressure; (c) CWAO experiments at 160 and 180ºC under stoichiometric or excess oxygen; (d) CWAO experiments at 180ºC, 7.6 bar of oxygen partial pressure at different initial pH values; (e) CWAO experiments at different NaCl concentrations. Initial PNP concentration: 5 g L^{-1}, 0.5 g of Ru/TiO_2.

The effect of P_{O2} was also almost negligible since the $\%COD_{RB}$ values were almost the same (Figure 5.9c). Nevertheless, there was an important increase of the unidentified intermediates fraction with the increase of P_{O2}.

The effect of pH on the biodegradability enhancement is shown on (Figure 5.9d). The highest fraction of biodegradable intermediates and, consequently, the highest $\%COD_{RB}$ was obtained at an initial pH of 4.6, which was the natural pH of the PNP solution without any pH adjustment. Nevertheless, the $\%COD_{RB}$ achieved at pH 2.0 and 8.0 were also high (40-50%), which means that these effluents were also clearly biodegradable. The most remarkable difference was the high fraction of unidentified intermediates obtained at an initial pH value of 2.0.

Finally, the presence of NaCl in high concentration in the reaction medium did not affect the performances of the CWAO process in terms of PNP and TOC conversions. However, the biodegradability decreased and the $\%COD_{RB}$ was reduced by 20% (Figure 5.9e). In this case, the difference could not be attributed to any change in the intermediates fraction, which were very similar (Figure 5.9e), but to a negative effect of NaCl over the biomass in the respirometric test (Kincannon and Gaudy, 1968).

In spite of the different results, all CWAO effluents obtained at 180ºC demonstrated a $\%COD_{RB}$ higher than 40%. This was a major result since the success of the biological treatment of industrial effluents with $\%COD_{RB}$ lower 25% has already been reported (Arslan and Ayberk, 2003; Suárez-Ojeda et al., 2007b). When selecting the most appropriate CWAO conditions for providing the most suitable effluent for a further biological treatment, it must be considered not only the PNP removal or TOC conversions, but also the intermediates distribution and, consequently the $\%COD_{RB}$. However, after evaluating all the operating parameters, if all of them meet the biodegradability requirements, the optimum conditions would be the one with the lowest running costs. From this study, it can be concluded, that the most suitable conditions would be to operate the oxidation at 180ºC under a stoichiometric P_{O2}. About the catalyst, there was no significant difference between the four catalysts, but Ru/TiO_2 seemed to be the best in terms of cost. Regarding the pH, there is no need of any pH adjustment before the

CWAO since no improvement was observed under acid or basic conditions. Finally, the impact of the saline content should be further studied to properly establish the impact on the CWAO process, and also over the biomass in the biological WWTP.

5.4. Conclusions

Several conditions were investigated for the WAO and CWAO of PNP. Conversion was higher than 90% for most of the tested conditions. Temperature was found to be the most important operating parameter upon CWAO, being the activation energy ca. 57 ± 1 kJ mol^{-1}. The PNP oxidation was not much influenced by the oxygen partial pressure. All the catalysts exhibited a similar behavior in terms of PNP and TOC conversions; however, the initial reaction rate was slightly higher over the platinum-based catalysts. The identification of the oxidation intermediates allowed establishing a reaction pathway proceeding through a free radical mechanism.

In relation to the biodegradability enhancement of the high-strength PNP wastewater, CWAO increased the biodegradability by more than 50% in most of the conditions tested. The carboxylic acid fraction was determined as a key factor to be taken into account, as the best biodegradability enhancement was observed when this fraction was the highest. The partial pressure of oxygen also had a negligible effect on the biodegradability enhancement. Even though the ionic strength did not affect the CWAO performance, the presence of NaCl decreased the effluent biodegradability. In terms of pH, the most suitable scenario was the one with no pH adjustment.

An integrated CWAO and biological treatment would certainly allow an easy removal of reaction intermediates formed during the first step of the treatment. The best CWAO conditions for such a pre-treatment of the high-strength PNP wastewater would be to work at 180ºC under a stoichiometric oxygen partial pressure (7.6 bar P_{O_2}) using a Ru/TiO_2 catalyst.

5.5. References

Adams, C., Cozzens, R., Kim, B. 1997. Effects of ozonation on the biodegradability of substituted phenols. *Water Research*, 31(10), 2655-2663.

Arslan, A., Ayberk, S. 2003. Characterisation and biological treatability of "Izmit industrial and domestic wastewater treatment plant" wastewaters. *Water Sa*, 29(4), 451-456.

Atwater, J.E., Akse, J.R., McKinnis, J.A., Thompson, J.O. 1997. Low temperature aqueous phase catalytic oxidation of phenol. *Chemosphere*, 34(1), 203-212.

Barbier-Jr, J., Delanoe, F., Jabouille, F., Duprez, D., Blanchard, G., Isnard, P. 1998. Total oxidation of acetic acid in aqueous solutions over noble metal catalysts. *Journal of Catalysis*, 177(2), 378-385.

Barbier-Jr, J., Oliviero, L., Renard, B., Duprez, D. 2002. Catalytic wet air oxidation of ammonia over M/CeO2 catalysts in the treatment of nitrogen-containing pollutants. *Catalysis Today*, 75(1-4), 29-34.

Barbier-Jr, J., Oliviero, L., Renard, B., Duprez, D. 2005. Role of ceria-supported noble metal catalysts (Ru, Pd, Pt) in wet air oxidation of nitrogen and oxygen containing compounds. *Topics in Catalysis*, 33(1-4), 77-86.

Bhatti, Z.I., Toda, H., Furukawa, K. 2002. p-Nitrophenol degradation by activated sludge attached on nonwovens. *Water Research*, 36, 1135-1142.

Bo, L., Quan, X., Chen, S., Zhao, H., Zhao, Y. 2006. Degradation of p-nitrophenol in aqueous solution by microwave assisted oxidation process through a granular activated carbon fixed bed. *Water Research*, 40(16), 3061-3068.

Béziat, J.C., Besson, M., Gallezot, P., Durecu, S. 1999. Catalytic wet air oxidation of carboxylic acids on TiO2-supported ruthenium catalysts. *Journal of Catalysis*, 182(1), 129-135.

Carrera, J., Martín-Hernández, M., Suárez-Ojeda, M., Pérez, J. 2011. Modelling the pH dependence of the kinetics of aerobic p-nitrophenol biodegradation. *Journal of Hazardous Materials*, 186(2-3), 1947-1953.

Castillejos-López, E., Maroto-Valiente, A., Nevskaia, D.M., Muñoz, V., Rodríguez-Ramos, I., Guerrero-Ruiz, A. 2009. Comparative study of support effects in ruthenium catalysts applied for wet air oxidation of aromatic compounds. *Catalysis Today*, 143, 355-363.

Collado, S., Laca, A., Diaz, M. 2011. Effect of the carboxylic substituent on the reactivity of the aromatic ring during the wet oxidation of phenolic acids. *Chemical Engineering Journal*, 166(3), 940-946.

Dai, Q., Lei, L., Zhang, X. 2008. Enhanced degradation of organic wastewater containing p-nitrophenol by a novel wet electrocatalytic oxidation process: Parameter optimization and degradation mechanism. *Separation and Purification Technology*, 61(2), 123-129.

Di Iaconi, C., Del Moro, G., De Sanctis, M., Rossetti, S. 2010. A chemically enhanced biological process for lowering operative costs and solid residues of industrial recalcitrant wastewater treatment. *Water Research*, 44(12), 3635-3644.

Eichenbaum, G., Johnson, D., Kirkland, P., O'Neil, P., Stellar, S., Bielawne, J., DeWire, R. 2009. Assessment of the genotoxic and carcinogenic risks of p-nitrophenol when is present as an impurity in a drug product. *Regulatory Toxicology and Pharmacology*, 55(1), 33-42.

EPER. 2004. Pollutant data from EU27 as in 2004. http://eper.ec.europa.eu/eper. Last time consulted 12/29/2011. *European Environment Agency. European Pollutant Emission Register*.

Gomes, H., Machado, B., Ribeiro, A., Moreira, I., Rosario, M., Silva, A., Figueiredo, J., Faria, J. 2008. Catalytic properties of carbon materials for wet oxidation of aniline. *Journal of Hazardous Materials*, 159(2-3), 420-426.

Grosjean, N., Descorme, C., Besson, M. 2010. Catalytic wet air oxidation of N,N-dimethylformamide aqueous solutions: Deactivation of TiO2 and ZrO2-supported noble metal catalysts. *Applied Catalysis B-Environmental*, 97(1-2), 276-283.

Jiang, Y., Zhu, X., Li, H., Ni, J. 2010. Effect of nitro substituent on electrochemical oxidation of phenols at boron-doped diamond anodes. *Chemosphere*, 78(9), 1093-1099.

Karrer, N., Ryhiner, G., Heinzle, E. 1997. Applicability test for combined biological-chemical treatment of wastewaters containing biorefractory compounds. *Water Research*, 31(5), 1013-1020.

Kim, K., Ihm, S. 2011. Heterogeneous catalytic wet air oxidation of refractory organic pollutants in industrial wastewaters: A review. *Journal of Hazardous Materials*, 186(1), 16-34.

Kincannon, D.F., Gaudy, A.F. 1968. Response of biological waste treatment systems to changes in salt concentrations. 10(4), 483 - 496.

Li, N., Descorme, C., Besson, M. 2008. Application of Ce0.33Zr0.63Pr0.04O2-supported noble metal catalysts in the catalytic wet air oxidation of 2-chlorophenol: Influence of the reaction conditions. *Applied Catalysis B-Environmental*, 80(3-4), 237-247.

Li, N., Descorme, C., Besson, M. 2007a. Catalytic wet air oxidation of 2-chlorophenol over Ru loaded CexZr1-xO2 solid solutions. *Applied Catalysis B-Environmental*, 76(1-2), 92-100.

Li, N., Descorme, C., Besson, M. 2007b. Catalytic wet air oxidation of chlorophenols over supported ruthenium catalysts. *Journal of Hazardous Materials*, 146(3), 602-609.

Liotta, L., Gruttadauria, M., Di Carlo, G., Perrini, G., Librando, V. 2009. Heterogeneous catalytic degradation of phenolic substrates: Catalysts activity. *Journal of Hazardous Materials*, 162(2-3), 588-606.

Liou, R., Chen, S., Huang, C., Lai, C., Shih, C., Chang, J., Hung, M. 2010. Catalytic wet peroxide oxidation of p-nitrophenol by Fe (III) supported on resin. *Water Science and Technology*, 62(8), 1879-1887.

Martín-Hernández, M., Carrera, J., Pérez, J., Suárez-Ojeda, M. 2009. Enrichment of a K-strategist microbial population able to biodegrade p-nitrophenol in a sequencing batch reactor. *Water Research*, 43(15), 3871-3883.

Mishra, K., Gogate, P. 2011. Intensification of sonophotocatalytic degradation of p-nitrophenol at pilot scale capacity. *Ultrasonics Sonochemistry*, 18(3), 739-744.

NLM. 1992. Hazardous Substances Data Bank: 4-Nitrophenol profile. http://toxmap.nlm.nih.gov/toxmap/main/chemPage.jsp?chem=4-Nitrophenol. Last time consulted: 27/04/2011. *U.S. National library of Medicine*.

OECD. 2008. The 2004 Organisation for Economic Co-operation and Development (OECD) List of High Production Volume Chemicals. http://www.oecd.org/dataoecd/55/38/33883530.pdf. Last time consulted: 26/04/2012, (Ed.) O.f.E.C.-o.a. Development.

Ogunbayo, T., Antunes, E., Nyokong, T. 2011. Investigation of homogeneous photosensitized oxidation activities of palladium and platinum octasubstituted phthalocyanines: Oxidation of 4-nitrophenol. *Journal of Molecular Catalysis a-Chemical*, 334(1-2), 123-129.

Oliviero, L., Barbier-Jr, J., Duprez, D. 2003a. Wet Air Oxidation of nitrogen-containing organic compounds and ammonia in aqueous media. *Applied Catalysis B-Environmental*, 40(3), 163-184.

Oliviero, L., Wahyu, H., Barbier-Jr, J., Duprez, D., Ponton, J.W., Metcalfe, I.S., Mantzavinos, D. 2003b. Experimental and predictive approach for determining wet air oxidation reaction pathways in synthetic wastewaters. *Chemical Engineering Research & Design*, 81(A3), 384-392.

Orupõld, K., Maširin, A., Tenno, T. 2001. Estimation of biodegradation parameters of phenolic compounds on activated sludge by respirometry. *Chemosphere*, 44(5), 1273-1280.

Pintar, A., Levec, J. 1994. Catalytic-Oxidation of Aqueous p-Chlorophenol and p-Nitrophenol Solutions. *Chemical Engineering Science*, 49(24A), 4391-4407.

Polo, A.M., Tobajas, M., Sanchis, S., Mohedano, A.F., Rodriguez, J.J. 2011. Comparison of experimental methods for determination of toxicity and biodegradability of xenobiotic compounds. *Biodegradation*, 22(4), 751-761.

Quintanilla, A., Casas, J., Rodriguez, J. 2010. Hydrogen peroxide-promoted-CWAO of phenol with activated carbon. *Applied Catalysis B-Environmental*, 93(3-4), 339-345.

Ricco, G., Tomei, M., Ramadori, R., Laera, G. 2004. Toxicity assessment of common xenobiotic compounds on municipal activated sludge: comparison between respirometry and Microtox (R). *Water Research*, 38(8), 2103-2110.

Santos, A., Yustos, P., Rodriguez, S., Garcia-Ochoa, F. 2006. Wet oxidation of phenol, cresols and nitrophenols catalyzed by activated carbon in acid and basic media. *Applied Catalysis B-Environmental*, 65(3-4), 269-281.

Suárez-Ojeda, M.E., Fabregat, A., Stuber, F., Fortuny, A., Carrera, J., Font, J. 2007a. Catalytic wet air oxidation of substituted phenols: Temperature and pressure effect on the pollutant removal, the catalyst preservation and the biodegradability enhancement. *Chemical Engineering Journal*, 132(1-3), 105-115.

Suárez-Ojeda, M.E., Guisasola, A., Baeza, J.A., Fabregat, A., Stuber, F., Fortuny, A., Font, J., Carrera, J. 2007b. Integrated catalytic wet air oxidation and aerobic biological treatment in a municipal WWTP of a high-strength o-cresol wastewater. *Chemosphere*, 66(11), 2096-2105.

Suárez-Ojeda, M.E., Stuber, F., Fortuny, A., Fabregat, A., Carrera, J., Font, J. 2005. Catalytic wet air oxidation of substituted phenols using activated carbon as catalyst. *Applied Catalysis B-Environmental*, 58(1-2), 105-114.

Tomei, M.C., Annesini, M.C. 2005. 4-Nitrophenol Biodegradation in a Sequencing Batch Reactor Operating with Aerobic−Anoxic Cycles. *Environmental Science Technology*, 39, 5059-5065.

Yang, S., Besson, M., Descorme, C. 2010. Catalytic wet air oxidation of formic acid over Pt/CexZr1-xO2 catalysts at low temperature and atmospheric pressure. *Applied Catalysis B-Environmental*, 100(1-2), 282-288.

Yang, S., Li, X., Zhu, W., Wang, J., Descorme, C. 2008. Catalytic activity, stability and structure of multi-walled carbon nanotubes in the wet air oxidation of phenol. *Carbon*, 46(3), 445-452.

Zhu, X., Ni, J., Li, H., Jiang, Y., Xing, X., Borthwick, A. 2010. Effects of ultrasound on electrochemical oxidation mechanisms of p-substituted phenols at BDD and PbO_2 anodes. *Electrochimica Acta*, 55(20), 5569-5575.

CHAPTER 6

6.1. General conclusions

The main objective of this thesis was to make a comparative study of several treatment strategies for the remediation of a high-strength PNP wastewater. Different approaches were considered with the aim of proposing a best available technique for the treatment of recalcitrant compounds at industrial level.

The treatment process selection not only depends on the nature of the recalcitrant compound but also its highly dependent on the compound(s) concentration, wastewater loading rate and influx scenario.

In this sense, biological treatment with acclimated activated sludge was proposed as the preferred choice for moderate to high PNP concentrations. To this end, a feeding strategy that prevents the substrate inhibition commonly associated to the biodegradation of PNP or recalcitrant compounds must be implemented. This fact was proven with the results achieved for more than three years of stable operation of the biological reactor treating PNP.

However, in real applications, biological treatment may be constantly facing shock loads, in the form of variations in the influent concentration, flow-rate or by the presence of toxic compounds that are not routinely treated in the wastewater plant. To overcome this problem a bioaugmentation strategy was implemented to enhance the operation of the endangered reactor, obtaining successful results.

Finally, for highly concentrated PNP wastewaters a combined chemical-biological treatment was proposed using CWAO with noble-metals based catalysts. In this case, complete mineralization of PNP was not pursued, but to obtain highly biodegradable effluents. This was done by examining the CWAO effluents produced under different operating conditions with respirometric techniques. Results from this study established the feasibility of coupling CWAO with a biological treatment to degrade highly concentrated

PNP wastewaters.

The overall results obtained in this research contributed to a deeper understanding of the dynamics of the degradation of PNP following different technologies and provides help in the decision making process for industrial wastewater remediation. The main conclusions drawn from this study are shown below:

- Biodegradation of PNP was possible in a SBR with a feeding strategy that was implemented for the enrichment of a K-strategist PNP-degrading activated sludge using conventional activated sludge from a municipal WWTP (i.e. non-acclimated biomass) as inoculum. The strategy was based on the distribution of the PNP addition along the aerobic reaction phase imposing endogenous conditions in 62% of the total reaction phase time of the SBR.
- Ninety-seven per cent of TOC removal and 100% of PNP elimination were achieved in the course of the whole operating period; with specific TOC loading rates between 0.13 to 0.38 g TOC g^{-1} VSS d^{-1} and with a maximum specific PNP loading rate of 0.26 g PNP g^{-1} VSS d^{-1}. No metabolic intermediates were detected.
- A mathematical model successfully describing the PNP degradation and the oxygen consumption was calibrated and validated using respirometric batch tests. The values obtained for the affinity constant for PNP (K_S) and the maximum specific PNP removal rate (k_{max}) allowed the biomass to be classified as a K-strategist and to corroborate the success of the applied feeding strategy.
- The value obtained for the substrate inhibition constant (K_i) is higher than that reported by most bibliographic references, meaning that the acclimated activated sludge of this work was more adapted to PNP inhibition than the remainder of the reported cultures.
- This PNP-degrading bacteria that is well adapted exhibiting high substrate affinity, is useful for bioaugmentation applications tested in this study.
- The key to the successful performance of a bioaugmented reactor under

transient shock loads is the survival of the PNP-degraders after several SRT without feeding PNP. In this sense, two factors were established as critical: the dosage of PNP-degraders and the use of the same co-substrate in both, the seeding and the seeded reactors. Bioaugmentation with PNP-degrading bacteria enhanced the removal of PNP on a SBR receiving transient or continuous PNP shock loads comparing to a non-bioaugmented SBR.

- A successful bioaugmentation process can be achieved with small dosages of PNP-degrading bacteria (2-5% w/w of total biomass in the seeded reactor). Such small dosages might allow a practical scale up for a real application in reactors treating high-strength wastewater.
- To treat a highly concentrated PNP wastewater, several conditions were investigated for WAO and CWAO, obtaining conversions higher than 90% for most of the tested conditions.
- Temperature was found to be the most important operating parameter upon CWAO, being the activation energy ca. 57 ± 1 kJ mol^{-1}, while PNP oxidation was not dependent on the oxygen partial pressure at the tested conditions.
- All the catalysts tested exhibited a similar behaviour in terms of PNP and TOC conversions; however, the initial reaction rate was slightly higher over the platinum-based catalysts.
- The identification of the oxidation intermediates allowed establishing a reaction pathway proceeding through a free radical mechanism.
- In relation to the biodegradability enhancement of the highly concentrated PNP wastewater, CWAO increased the biodegradability by more than 50% in most of the conditions tested. The carboxylic acid fraction was determined as a key factor to be taken into account, as the best biodegradability enhancement was observed when this fraction was the highest.
- An integrated CWAO and biological treatment would certainly allow an easy remediation of this type of industrial wastewaters.

6.2. Future work

This thesis had the aim of providing a wide assessment on the best technique to deal with wastewaters contaminated with PNP, a recalcitrant compound. It was demonstrated that acclimated biomass is effective for obtaining complete PNP removal from wastewater. Nevertheless, in this thesis the focus was to obtain a biomass with a high substrate affinity and to reduce the inhibition problems related to PNP biodegradation. In this sense, future research on this matter should be directed to optimize the SBR working conditions to treat higher PNP loads by using granulated or immobilized biomass.

Also, additional studies should be made to reduce the concentration of glucose used as co-substrate to determine if a stable operation on the SBR could be achieved using PNP as a sole carbon source. Moreover, real industrial wastewater often contains a wide variety of compounds that are complex to treat with biological treatments. In this scenario, the study of simultaneous PNP degradation along with other phenolic compounds is proposed. Finally on this matter, it is advised to study simultaneous PNP degradation and denitrification by optimizing the SBR conditions introducing anoxic cycles to favour the development of denitrifying bacteria.

Regarding bioaugmentation, it is a promising tool for enhancing the performance of biological reactors that are submitted to toxic shock loads or to reduce the start up time of a biological reactor. The key parameters for the process are the dosage and the survival of the specialized biomass introduced on this matter, further studies should be conducted to optimize those parameters. Immobilizing the specialized sludge in the seeded reactor or introducing it as granular sludge might help overcome the survival and the dosage problem by preventing the bacteria washout from the reactor.

Finally, regarding to the treatment of highly concentrated PNP wastewaters, it was demonstrated that CWAO increased the biodegradability of these wastewaters and reduced the toxicity effect for urban WWTP sludge; however, the urban WWTP sludge was not fully capable to degrade

recalcitrant compounds left on CWAO effluents. To attain this matter, further studies with acclimated biomass for total COD removal of CWAO effluents should be done. An optimization of combined chemical and biological process might be done using the intermediates distribution on the course of CWAO reactions, so the CWAO reaction time could be reduced. In this stage, economical studies should be implemented to find the optimum operation parameters.

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Education:

- **Doctorate in Environmental Science and Technology.** Universitat Autònoma de Barcelona. Barcelona – Spain. (Ongoing Studies). Awarded with the prestigious "Mención de Calidad" for quality education in doctoral programs by the Spanish Ministry for Education (MEE 2011-0443). "Thesis title: Comparative study on the treatment of a high-strenght *p*-nitrophenol wastewater".
- **Master in Environmental Studies;** specialized in Environmental Technology Universitat Autònoma de Barcelona. Barcelona – Spain. June 2008
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- **Teaching assistant** at the Department of Chemical Engineering at the Universitat Autònoma de Barcelona. Subjects related with chemical engineering degree: industrial chemistry: industrial security, chemical engineering laboratory. Subjects related with technical in industrial engineering degree: water treatment technologies. Subjects related with Environmental science degree: basic operations in chemistry laboratory. October 2007 – October 2011.
- **Research stay** in the Water and Wastewater Treatment research team at the Institut de Recherches sur la Catalyse et l'Environnement de Lyon in the research line of oxidation of pollutants through catalytic wet air oxidation. Lyon – France. February 2010 – July 2010.
- **Applications Engineer**. Commercial equipment and systems division of Hidrocaven CA. Job description: expert advice and customer support in the wastewater treatment department; in addition, consulting on products related to water management. Caracas – Venezuela. November 2006 – April 2007

- **Intern**. Internship in process validation in the maintenance department at FARMA S.A. Laboratories. Job description: Identification of critical variables and technique evaluation to the instrumentation in the manufacturing plant of solid and granular products at Laboratorios FARMA S.A. Caracas – Venezuela. July 2005 – December 2005

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- **Mariángel Martín-Hernández**, Julián Carrera, María Eugenia Suárez-Ojeda, Michèle Besson, Claude Descorme. (2012). Catalytic wet air oxidation of a high strength p-nitrophenol wastewater over Ru and Pt catalysts: Influence of the reaction conditions on biodegradability enhancement**.** Applied Catalysis B: Environmental Volume: 123-124 Pages: 141-150.
- María Eugenia Suárez-Ojeda, Helena Montón, Mónica Roldán, **Mariángel Martín-Hernández,** Julio Pérez, Julián Carrera. (2011). Characterization of a p-nitrophenol-degrading mixed culture with an improved methodology of fluorescence in situ hybridization and confocal laser scanning microscopy. Journal of Chemical Technology and Biotechnology Volume: 86 Issue: 11 Pages: 1405-1412.
- Julián Carrera, **Mariángel Martín-Hernández**, María Eugenia Suárez-Ojeda, Julio Pérez. (2011). Modelling the pH dependence of the kinetics of aerobic p-nitrophenol biodegradation. Journal of Hazardous Materials Volume: 186 Issue: 2-3 Pages: 1947-1953
- **Mariángel Martín-Hernández**, Julián Carrera, Julio Pérez, María Eugenia Suárez-Ojeda. (2009). Enrichment of a K-strategist microbial population able to biodegrade p-nitrophenol in a sequencing batch reactor. Water Research Volume: 43 Issue: 15 Pages: 3871-3883

Participation on funded projects

- Project title: Treatment of complex industrial wastewaters through completely biological process using aerobic reactors with granular biomass (ONLYBIO).

  Funding entity: CICYT nº CTQ2011-24745/PPQ. From January 2012 to December 2014

  Participating entities: Department of Chemical Engineering (Universitat Autònoma de Barcelona)

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- Project title: Application of aerobic reactors with granular sludge to urban wastewaters: pilot scale operation and mathematical modeling.

  Funding entity: CICYT nº CTQ2008-06792-C02-02/PPQ. From January 2009 to December 2011

  Participating entities: Departamento de Ingeniería Química (Universitat Autònoma de Barcelona). Departamento de Ingeniería Química (Universidade de Santiago de Compostela)

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- Congress: 15th European Microscopy Congres (emc2012). Manchester – United Kingdom. September 2012.

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- Congress: 8th World Congress of Chemical Engineering. Montreal – Canada. September 2009.

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- Participation and adoption of technical course "Management of Chemicals" in the framework of the Sixth National Congress of Students in Chemical Engineering (October 2002). La Universidad del Zulia. Maracaibo. Venezuela.
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