NOVEL ANAEROBIC PACKED-BED REACTOR APPLICATION FOR WASTEWATER REMEDIATION

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FAIG CONSTAR que aquest treball, titulat "Novel anaerobic packed-bed reactor application for wastewater remediation", que presenta Yonhara García Martínez per a l'obtenció del títol de Doctor, ha estat realitzat sota la meva direcció al Departament d' Enginyeria Química d'aquesta universitat.

HAGO CONSTAR que el presente trabajo, titulado "Novel anaerobic packed-bed reactor application for wastewater remediation", que presenta Yonhara García Martínez para la obtención del título de Doctor, ha sido realizado bajo mi dirección en el Departamento de Ingeniería Química de esta universidad.

I STATE that the present study, entitled "Novel anaerobic packed-bed reactor application for wastewater remediation", presented by Yonhara García Martínez for the award of the degree of Doctor, has been carried out under my supervision at the Department of Chemical Engineering of this university.

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Els directors de la tesi doctoral
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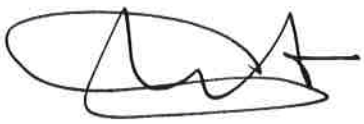

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DOCTORAL THESIS

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Resumen

Los contaminantes emergentes son compuestos que presentan distinta naturaleza química y origen, siendo su presencia en el medioambiente y las consecuencias de esta no precisamente nuevas, pero solo tenidas suficientemente en cuenta hace unas décadas. Entre los compuestos emergentes podemos encontrar fármacos, pesticidas, compuestos aromáticos, colorantes, etc., y se encuentran dispersados en ambientes acuáticos marinos y terrestres. Son compuestos de los cuales actualmente no se conoce mucho acerca de su presencia e impacto al medio ambiente y la salud humana, pero sí se ha establecido que la principal vía de entrada de estos compuestos en el ambiente acuático son las aguas residuales urbanas e industriales. Actualmente no existen procedimientos establecidos para su regulación y eliminación de las aguas, ya que debido a su toxicidad, diversidad y variación en la concentración son difícilmente eliminadas en las plantas depuradoras convencionales.

En las últimas décadas, las investigaciones se han centrado en evaluar la eficiencia de nuevas tecnologías para la eliminación de disímiles contaminantes emergentes presentes en las aguas residuales, con el propósito de sugerir alternativas que posibiliten disminuir la presencia de estos contaminantes a un bajo coste ambiental, económico y energético. Los tratamientos biológicos se presentan como la mejor alternativa, ya que convierten la materia orgánica acuosa en biomasa y son económicos; aunque en ocasiones la mineralización que se obtiene del contaminante no es completa y por tanto, se requiere de tratamientos posteriores que eliminen los compuestos orgánicos remanentes en los efluentes.

Por tanto, este trabajo de tesis se ha centrado en la utilización de un reactor anaerobio agitado de lecho empacado de flujo ascendente (USPBR) para eliminar nitratos, clorobenceno (CB), ácido 2,4-diclorofenoxiacético (2,4-D), ibuprofeno (IBP) y el colorante Acid Orange 7 (AO7) de soluciones acuosas. La investigación se centró en estudiar la adsorción de algunos de estos contaminantes sobre el carbón activado (AC) y su reducción mediante el empleo de cultivos inmovilizados sobre AC y/o material carbonoso a base de lodos (SCM).

Los resultados de la remoción de nitratos en un USPBR formado por un cultivo anaerobio inmovilizado sobre SCM (BSCM), muestran un alto grado de conversión de nitratos, hasta un 99%, a muy cortos tiempos espaciales (τ), 2minutos, correspondiendo a tiempos de residencia hidráulicos (HRT) de aproximadamente 6 minutos. Del estudio de la velocidad de denitrificación y la remoción de nitratos en función de la concentración en el USPBR con SCM en comparación con un USPBR con AC, se obtuvieron valores un poco superiores en el sistema con SCM. Además, los resultados del estudio cinético demostraron que el modelo de Michaelis-Menten describe bien el proceso de

denitrificación a bajas concentraciones. Por lo que, el SCM demostró ser un catalizador económico potencial y que compite eficazmente con los comerciales.

Posteriormente, se estudió la adsorción del CB, 2,4-D e IBP sobre el carbón activado, así como la biodegradación de estos contaminantes en el USPBR formado por un cultivo anaerobio inmovilizado sobre AC (BAC). Los resultados muestran que la capacidad de adsorción sigue el orden IBP<2,4-D<CB y que el empleo de una agitación lenta en el lecho de carbón proporcionó un incremento de la bioconversión de CB, IBP y 2,4-D de alrededor del 90 % a τ de 0,6, 1 y 1,2 h, respectivamente.

Los resultados de la conversión de CB se ajustaron al modelo de Michaelis-Menten, sin embargo para el 2,4-D e IBP fue necesario expandir dicha cinética con un factor de inhibición que produjo un modelo que ajusta bien los datos experimentales; indistintamente de la concentración inicial del contaminante.

Además, en el presente trabajo la preparación, modificación de la química superficial y la caracterización de materiales a base de carbono, como son AC, nanotubos de carbono (CNTs) y xerogeles (CXs), se llevó a cabo con el propósito de investigar en futuros trabajos, su influencia en las velocidades de reducción de los contaminantes estudiados. Los resultados indican que el tratamiento de oxidación con ácido nítrico produjo materiales de carbonos más ácidos y con altas cantidades de grupos de oxígeno en su superficie, aunque las muestras sometidas a tratamientos térmicos presentaron un carácter más básico. Mientras que las muestras tratadas con urea y melanina, utilizados como precursores de nitrógeno, aumentaron la basicidad superficial de los materiales y por tanto, pueden intensificar la interacción con moléculas de ácido.

Por último, la mineralización completa del AO7 se estudió en un sistema integrado USPBR-BSCM con un reactor aerobio de membrana. Los resultados han demostrado una vez más que el SCM es un buen material catalítico para el sistema anaerobio, obteniéndose altas velocidades de conversión del AO7 a τ muy pequeños, alrededor del 99 % a 1,02-1,6 minutos. Los productos de la reducción fueron completamente mineralizados en la posterior etapa aerobia. Las aminas aromáticas y la demanda química de oxígeno fueron degradadas en dicha etapa a bajos HRT. La eficiencia de remoción total de aminas aromáticas del sistema anaerobio USPBR-BSCM/aerobio de membrana fue de 80,28 %.

Summary

The emergent pollutants (EPs) are compounds with different chemical nature and origin, being its presence in the environment and the consequences of these no precisely new, but just sufficiently taken into account a few decades ago. In the EPs we can find pharmaceuticals, pesticides, aromatic compounds, azo dyes, etc., and are found dispersed in aquatic environments, marine and terrestrial. They are compounds that currently little is known about their presence and impact to the environment and human health, but it has been established that the main way to entrance of these pollutants in the aquatic environment are urban and industrial wastewaters. Nowadays, there are no stated procedures for their regulation and removal of waters, due to their toxicity, diversity and variation in the concentration are difficult to be degraded in conventional wastewaters treatment plants (WWTPs).

In the last few decades, research have focused on the assessment the efficiency of new technologies for the removal of different EPs present in wastewaters, for the purpose of suggest alternatives that enable to reduce the presence of these contaminants to a low ambient, economic and energetic cost. The biological treatments appears to be the better option, because of they convert the aqueous organic matter to biomass and their cost-effective; though, sometimes the mineralization of the pollutant is not complete and therefore, post-treatments are required that removed remain organic compounds in the effluents.

Thus, this thesis has been focused in the utilization of an anaerobic up-flow stirred packed-bed reactor (USPBR) for the removal of nitrates, chlorobenzene (CB), 2,4-dichlorophenoxyacetic acid (2,4-D), ibuprofen (IBP) and Acid Orange 7 (AO7) of aqueous solutions. The research focused in the study of the adsorption of some of these contaminants over activated carbon (AC) and their reduction using immobilized cultures over AC and/or sludge carbonaceous material (SCM).

The results of the nitrate removal in USPBR filled with biological sludge carbonaceous material (BSCM), showed a high conversion values, up to 99 %, at very short space times (τ), 2 min, corresponding to hydraulic residence times (HRT) of about 6 min. The denitrification rate and removal rate were studied as a function of nitrate concentration in USPBR-SCM compared with USPBR-AC, values a little high in the system with SCM were obtained. Moreover, the results of the kinetic study demonstrated that the Michaelis-Menten model adequately described the denitrification process at low concentrations. Hence, the SCM proved to be a potentially low cost catalyst and efficiently competes with the commercial ones.

Subsequently, the adsorption of CB, 2,4-D and IBP over AC were studied, as well as the biodegration of these pollutants in USPBR filled with BAC. The

results revealed that the adsorption capacity follow the order IBP<2,4-D<CB and the employment of an slow agitation in the biological bed provided an increment of CB, IBP y 2,4-D bioconversion of about 90 % at τ of 0,6, 1 y 1,2 h, respectively.

The experimental results of CB has a good fitting to the Michaelis-Menten model, however, for 2,4-D and IBP was necessary to expand the model with an inhibition factor, that produced a well-fitting to the experimental data; indistinctly of the pollutant initial concentration.

In addition, in the present work the preparation, surface chemistry modification and characterization of carbon based materials, such as AC, carbon nanotubes (CNTs) and carbon xerogels (CXs), were carried out with the purpose to investigate in future works, their influence in the reaction rates of the studied compounds. The results suggest that the oxidation treatment with nitric acid (HNO3) produced more acid carbon based materials and with high quantity of oxygen groups on their surface, although the samples heat treated showed a more basic character. While the samples treated with urea and melamine, used as nitrogen precursors, increased the surface basicity of the carbon materials and therefore, can enhance the interaction with acid molecules.

Finally, the complete mineralization of AO7 was studied in an integrated system USPBR-BSCM with an aerobic membrane bioreactor (aerobic MBR). The results have shown once again that the SCM is a good catalytic material for the anaerobic treatment, obtaining high AO7 conversion values at very short τ, up to 99 % at 1,02-1,6 min. The by-products reduction was completely removed in the subsequent aerobic stage. The aromatic amines and the chemical oxygen demand (COD) were degraded in this stage at low HRTs. The total aromatic amines (TAA) removal efficiency of the anaerobic USPBR-BSCM/ aerobic MBR was about 80,28 %.

Resum

Els contaminants emergents són compostos que presenten diferent naturalesa química i origen, sent la seva presència en el medi ambient i les conseqüències d'aquesta no precisament noves, però només tingudes prou en compte fa unes dècades. Entre els compostos emergents podem trobar fàrmacs, pesticides, compostos aromàtics, colorants, etc., i es troben dispersats en ambients aquàtics marins i terrestres. Són compostos dels quals actualment no es coneix molt sobre la seva presència i impacte al medi ambient i la salut humana, però sí que s'ha establert que la principal via d'entrada d'aquests compostos en l'ambient aquàtic són les aigües residuals urbanes i industrials. Actualment no hi ha procediments establerts per a la seva regulació i eliminació de les aigües, ja que a causa de la seva toxicitat, diversitat i variació en la concentració són difícilment eliminats en les plantes depuradores convencionals.

En les últimes dècades, les investigacions s'han centrat en avaluar l'eficiència de noves tecnologies per a l'eliminació de diversos i contaminants emergents presents en les aigües residuals, amb el propòsit de suggerir alternatives que possibilitin disminuir la presència d'aquests contaminants a un baix cost ambiental, econòmic i energètic. Els tractaments biològics es presenten com la millor alternativa, ja que converteixen la matèria orgànica aquosa en biomassa i són econòmics; encara que en ocasions la mineralització que s'obté del contaminant no és completa i per tant, es requereix de tractaments posteriors que eliminin els compostos orgànics romanents en els efluents.

Per tant, aquest treball de tesi s'ha centrat en la utilització d'un reactor anaerobi de flux ascendent reactor agitat de llit empacat (USPBR) per eliminar nitrats, clorobenzè (CB), àcid 2,4-diclorofenoxiacètic (2,4-D), ibuprofèn (IBP) i el colorant taronja àcid juliol (AO7) de solucions aquoses. La investigació es va centrar en estudiar l'adsorció d'alguns d'aquests contaminants sobre el carbó activat (AC) i la seva reducció mitjançant l'ús de cultius immobilitzats sobre AC i/o material carbonós (SCM).

Els resultats de la remoció de nitrats en un USPBR format per un cultiu anaerobi immobilitzat sobre SCM (BSCM), mostren un alt grau de conversió de nitrats, fins a un 99%, a molt curts temps espacials (τ), 2 minuts, corresponent a temps de residència hidràulics (HRT) d' aproximadament 6 minuts. De l'estudi de la velocitat de desnitrificació i la remoció de nitrats en funció de la concentració en el USPBR amb SCM en comparació amb un USPBR amb AC, es van obtenir valors una mica superiors en el sistema amb SCM. A més, els resultats de l'estudi cinètic van demostrar que el model de Michaelis-Menten descriu bé el procés de desnitrificació a baixes concentracions. Pel que, el SCM va demostrar ser un catalitzador econòmic potencial i que competeix eficaçment amb els comercials.

Posteriorment, es va estudiar l'adsorció del CB, 2,4-D i IBP sobre el carbó activat, així com la biodegradació d'aquests contaminants en el USPBR format per un cultiu anaerobi immobilitzat sobre AC (BAC). Els resultats mostren que la capacitat d'adsorció segueix l'ordre IBP <2,4-D <CB i que l'ocupació d'una agitació lenta en el llit de carbó va proporcionar un increment de la bioconversió de CB, IBP i 2,4-D de voltant del 90% a τ de 0,6, 1 i 1,2 h, respectivament.

Els resultats de la conversió de CB es van ajustar al model de Michaelis-Menten, però per al 2,4-D i IBP va ser necessari expandir aquesta cinètica amb un factor d'inhibició que va produir un model que ajusta bé les dades experimentals; indistintament de la concentració inicial del contaminant.

A més, en el present treball la preparació, modificació de la química superficial i la caracterització de materials a base de carboni, com són AC, nanotubs de carboni (CNT) i xerogeles (CXS), es va dur a terme amb el propòsit d'investigar en futurs treballs, la seva influència en les velocitats de reducció dels contaminants estudiats. Els resultats indiquen que el tractament d'oxidació amb àcid nítric produir materials de carbonis més àcids i amb altes quantitats de grups d'oxigen en la seva superfície, tot i que les mostres sotmeses a tractaments tèrmics van presentar un caràcter més bàsic. Mentre que les mostres tractades amb urea i melamina, utilitzats com a precursors de nitrogen, van augmentar la basicitat superficial dels materials i per tant, poden intensificar la interacció amb molècules d'àcid.

Finalment, la mineralització completa del AO7 es va estudiar en un sistema integrat USPBR-BSCM amb un reactor aerobi de membrana. Els resultats han demostrat una vegada més que el SCM és un bon material catalític per al sistema anaerobi, obtenint altes velocitats de conversió AO7 a τ molt petits, al voltant del 99% a 1,02-1,6 min. Els productes de la reducció van ser completament mineralitzats en la posterior etapa aeròbia. Les amines aromàtiques i la demanda química d'oxigen (COD) van ser degradades en aquesta etapa a baixos HRT. L'eficiència de remoció total d'ammines aromàtiques del sistema anaerobi USPBR-BSCM / aerobi de membrana va ser de 80,28%.

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Chapter 1

Introduction

1.1. Description and relevance of the topic

In the latest decenniums, the world has experienced the negative effects of unrestrained growth of diverse human activities, such as industry, transport, agriculture and urbanization. The increment in living standards and the rising customer demand has increased the water pollution with the so-called “emerging pollutants” (EP), which has converted in an interest growing area for environmental researchers. Due to the possible impacts of these substances for environment and human health, even at very low concentrations (ng L^{-1} range) (Gavrilescu et al. 2014).

There is no internationally agreed definition for an emerging pollutant (EP) and a number of definitions have been proposed, but a misconception that we can manage is that EPs are not necessarily new chemicals; they may be substances that have been present in the environment for a long time but whose presence and significance are only now being recognized (Halden 2007).The terminology has come to include a broad variety of man-made chemicals, for example dyes, pesticides, pharmaceuticals and personal care products (PPCPs), chlorinated aromatic compounds, metals, endocrine disrupting compounds, among others; which are in use across the world and are essential for modern day society. A general characteristic to all of them is that they are not incorporated in habitual control programs at global level, though have been added to the Candidates Contaminant List for prioritizing their regulation in the near future (Carvalho et al. 2013; USEPA 2009; Council 2008).

It has been shown that between 1930 and 2000, the worldwide production of chemicals ascended from 1 million to 400 million tons per each year. Stats reported by EURO-STAT in 2013 disclose that, between 2002 and 2011, more than 50% of the total production of chemicals is portrayed by environmentally injurious compounds and over 70% of these are chemicals with significant environmental impact (Gavrilescu et al. 2014).

The effects associated with the appearance of EPs on environment and human health are very diverse, for example, dyes tend to bio accumulate in the environment, and have carcinogenic, allergenic and mutagenic characteristics for human being (Gudelj et al. 2011). In the case of chlorinated aromatic compounds, the most common toxicity effects in animal species include weight loss, impaired liver function, general malaise, hepatic porphyria, peripheral neuropathies and pathological changes in the skin (Greenlee et al. 1985).

The manner of the EPs to enter into the environment depends on their use and mode of application, for example, through industrial and agricultural wastes, discharge of municipal, excretion of pharmaceuticals, etc. (Farré et al. 2008). The conventional wastewater treatment plants are not designed to remove all EPs, due to they generally eliminate organics and pathogens; some of these EPs can linger even after a tertiary wastewater process, and may be inserted again into the environment when the effluent is discharged to surface water, recharged into groundwater or remain in the treated sewage sludge (Raghav et al. 2013).

Nowadays, investigations have been focused on the effective treatment to lower the impact of contaminated wastewater effluents. Currently, available technologies for the efficient removal of micropollutants from wastewater are based on physical, chemical, electrochemical and biological methods (Bazrafshan et al. 2013; Farré et al. 2001; García-Martínez et al. 2015; Mailler et al. 2014; Vieira et al. 2011). Generally, physical, chemical and electrochemical treatment technologies present technical and economical limitations when are applied alone at large scale; thus, indubitably biological processes seem to be the most inexpensive and environmentally suitable treatment (Girardi et al. 2013; González et al. 2006; Liang et al. 2013; Samaras et al. 2014).

Another important type of pollutants is the inorganic nitrogen compounds; in the aquatic ecosystems the more common ionic forms of inorganic nitrogen are ammonium, nitrite and nitrate. They can be present in the aquatic environment in a natural way, as a consequence of the biological degradation of organic matter, dissolution of nitrogen-rich geological deposits, atmospheric deposition, surface and groundwater runoff, among others (Camargo et al. 2005). Consequently, the concentration of nitrate in marine environment and fresh-water are frequently greater than those of ammonium and nitrite (Rabalais 2002).

On the other hand, these inorganic nitrogen compounds are deemed undesirable substances in water; despite they are produced in the water naturally, the presence of high concentration levels in groundwater are

commonly a consequence of the human activities, for example, in chemical fertilizers in agriculture and inadequate disposal of animal and human wastes. They also have been detected in groundwater reserves and surface waters, such as rivers and lakes; because of the increment in the agriculture, urbanization and industrialization (Tavares de Sousa et al. 2009; Montalvo et al. 2014).

The high levels of inorganic nitrogen pollutants can cause an acidification and eutrophication in the aquatic ecosystems, endangering the capacity of the aquatic animals to survive, growth and reproduce (Luo et al. 2016). In human health, can provoke cancer in the digestive tract, bladder and ovarian, induce the methemoglobinemia in young infants, contribute to non-Hodgkin's lymphoma or cause spontaneous abortions, among others (Camargo & Alonso 2006).

The processes developed for removing inorganic nitrogen compounds from water and wastewaters, can be classified in two main groups: physicochemical and biological methods (Bravo et al. 2013; Yu et al. 2013; Andalib et al. 2012). The physicochemical methods do not solve the problem totally, due to they just transferred the contaminant from one matrix to another and are not profitable; whilst, biological treatments remove the pollutant and operating under proper conditions the final products are harmless to the environment, and by-products can be obtained to use them as fertilizers (Yetilmezsoy & Sapci-Zengin 2009; Uysal et al. 2010).

In the present work, AO7, 2,4-dichlorophenoxy acetic acid (2,4-D), chlorobenzene (CB), ibuprofen (IBP) and nitrate, were taking as target pollutants to study the removal of EPs and inorganic nitrogen contaminants from aqueous solution.

Acid Orange 7 (AO7) belongs to the chemical group of azo dyes, which are substances commonly used in textile, pharmaceutical, and food industries and characterized by the N=N bond. Their production is more than 1 million tons per year in the world, and during dying processes, about 40% of this huge amount of azo dyes ends up in wastewaters (Manu & Chaudhari 2002); constituting a serious menace for human health. It is reported that AO7 is extremely toxic and the ingestion can provoke eye, skin and mucous membrane irritation, dermatitis, loss of bone marrow, severe headaches, among others (Gupta et al. 2006).

The dyes have been prepared to be resistant to chemical composites (soaps and detergents), sunlight and all agents that can enter in touch with the product. Nevertheless, as more resistant is the dye to these agents, more difficult is to remove the colour from wastewaters (Méndez-Paz et al. 2005). There is no adequate process to treat these wastewaters at high concentrations and at soft conditions on the industrial scale for the time being, therefore, the development of treatment methods for the decomposition and removal of AO7 and its degradation products, in wastewater is imperatively required.

Nitrate is an inorganic nitrogen compound, as we mentioned before, and is employed principally in inorganic fertilizers, in the production of explosives, as

an oxidizing agent and for glass making; it is also naturally occur in plants, being a fundamental nutrient (World Health Organization 2011). The excess of nitrates in water can produce, as principal health diseases, the methemoglobinemia, carcinogenesis and maternal exposure to environmental nitrates may increase the risk of pregnancy problems like anaemia, threatened abortion/premature labour, or preeclampsia (Agency for Toxic Substances & Disease Registery 2013).

There are several treatments to remove nitrates from water and wastewaters, but the most commonly used are adsorption (Rajeswari et al. 2016), ion exchange (Nur et al. 2015), reverse osmosis (Schoeman & Steyn 2003), electro-dialysis (Sahli et al. 2004), catalytic (Hamid et al. 2015) and biological methods (Mohseni-Bandpi et al. 2013).

2,4-dichlorophenoxy acetic acid (2,4-D) is one of the most applied herbicides around the world to control perennial broad-leaved weeds in cereal cropland, pastures, forests and to control broad-leaved aquatic weeds (Bazrafshan et al. 2013). However, it is toxic to broad-leafed plants due to its polar nature and once absorbed by the plant it accumulates at the growing points of roots; inhibiting the growth of the plant (Shankar et al. 2006). In addition, the exposure of animal species to this herbicide can affect the central nervous (Bortolozzi et al. 2004).

Several methods have been examined for 2,4-D removal from water and wastewaters, such as photo catalytic process (Chu et al. 2004), advanced

oxidation (Bandala et al. 2007), electrochemical oxidation (Gao et al. 2009), biological treatment (Santacruz et al. 2005), ion exchange (Kwan & Chu 2006), activated carbon adsorption (Chingombe et al. 2006), and other adsorbents (Zhou et al. 2011), among others.

Chlorobenzene is a chlorinated aromatic compound and is commercially produced by the chlorination of benzene in the presence of a catalyst. It is a flammable liquid and is used as an industrial solvent, in the production of nitrochlorobenze and intermediates, for the synthesis of dyes, pharmaceuticals, and products for the rubber and plastic industries (Committee on Acute Exposure Guideline Levels et al. 2012; Malcolm et al. 2004; Oonnittan et al. 2009; Braeckevelt et al. 2011). Its production, use and inadequate removal leads with their presence in groundwater, surface water and soil samples, implying serious threats to the public health and drinking water security (Field & Sierra-Alvarez 2007; Ma et al. 2012; Chary et al. 2012; Wang et al. 1992).

A wide number of research have been made to search an efficient technology for chlorobenzene degradation, such as, adsorption (Sennour et al. 2009), catalytic oxidation (Hofmann et al. 2005), photo catalysis (Oghenejoboh et al. 2013), non-thermal plasma oxidation (Karuppiah et al. 2013) and biological filtration (Li et al. 2014).

Ibuprofen (IBP) is an antipyretic product and has been classified as a non-steroidal anti-inflammatory drug; it is widely used in the treatment of rheumatic disorders, pain and fever (Mestre et al. 2007). Nevertheless, IBP is one of the

important drugs included in the ‘‘Essential Drugs List’’ of World Health Organization (Heckmann et al. 2007), because of its widespread use and persistence into the environment. Moreover, it is also considered to be one of the most important pharmaceutical contaminants in sewage treatment plant influents (Winkler et al. 2001).

Diverse technologies have been proposed in removing ibuprofen from wastewater effluents; such as, adsorption processes (Guedidi et al. 2014), advanced oxidation processes (Schrantz et al. 2013), membrane filtration (Vergili 2013) and electrochemical oxidation (Nikolić-Bujanović et al. 2016). However, these processes are not “eco-friendly” (Wenzel et al. 2008; Høibye et al. 2008) and wastewaters treatment plants (WWTPs) are equipped only for physical processes and nutrient removal, due to the high cost of advanced sewage treatments (Kosma et al. 2010; Gupta et al. 2012; Reungo at et al. 2011).

As can be seen, the EPs selected and nitrates are difficult to be degraded under natural conditions and in general, the physical, chemical and electrochemical technologies seem to face the facts of technical and economical limitations. Thus, the research of the actual literature confirm that the biological treatments are the most attractive, considering its economic, environmentally suitable and methodologically relatively simple features (González et al. 2006; Samaras et al. 2014; Girardi et al. 2013).

Investigations on the biodegradability of this kind of micropollutants under aerobic conditions are well documented, but only few authors have studied the degradation under anaerobic conditions; there are several reasons why anaerobic biodegradation processes appear to be the better option. Anaerobic treatments are technologically simple and relatively cheap; furthermore, they consume little energy. Anaerobic microorganisms can be conserved unfed during long periods without any severe decline of their activity. For anaerobic treatments the nutrient need is low and it is least tender to toxic substances (Savant et al. 2006).

Recently, biofilms have become a focus of interests for the researchers in the field of biodegradation of EPs and nitrates. Biofilms have been found to be suitable for the remediation of pollutants because of their high microbial biomass and ability to immobilize pollutants (Daset. al 2012). Immobilized bacteria have been proved to be more effective than suspended bacteria for biodegradation of these recalcitrant pollutants (Andersson et al. 2008). Nevertheless, the reduction of numerous recalcitrant pollutants take place pretty slowly, that leads to low yield of bioreactors (Van der Zee et al. 2001). Therefore, the necessity to use redox mediators, which are compounds that can be reversibly oxidised and reduced, serving as electron carriers in many redox reactions and increasing the reaction rate by one or more orders of magnitude (Van der Zee & Cervantes 2009).

Activated carbon (AC) has been proven to be an effective redox mediator for the removal of a wide variety of micropollutants and as an inert porous carrier

material, it is capable of distributing chemicals on its large hydrophobic internal surface, thus making them accessible to reactants (Yin et al. 2007). However, the use of sewage sludge based carbon materials, carbon nanotubes (CNT) and carbon xerogels (CXs) as catalysts for micropollutants degradation has been demonstrated by (Athalathil et al. 2014; Gonçalves et al. 2010; Orge et al. 2012), respectively. The new mesoporous materials can present technological advantages as new catalysts principally for the EPs degradation (Pereira et al. 2014); but little data are available in the literature on the removal of the selected EPs from contaminated water using the combination of sludge and these new materials.

Previous studies developed in the research group, have shown high reduction of azo dyes, around 99% at very short space time (τ), 2.0 min (Mezohegyi et al. 2007; Athalathil et al. 2014). They essayed continuous operation in packed-bed type reactors in an up-flow mode, containing activated carbon (AC) or sludge carbonaceous material (SCM) with an immobilized anaerobic mixed culture. The biological activated carbon (BAC) or biological sludge carbonaceous material (BSCM) processes utilizes granular activated carbon (GAC) or granular carbonaceous materials (GCM) to adsorb organics and to act as a support for microorganisms (biofilm), thus facilitating the elimination of organic water pollutants (Zhang & Yu 2000). The studies reported that the anaerobic, catalytic reductive azo-dye degradation technique, using BAC or BSCM, is an effective and promising treatment for the effective azo reduction.

On the other hand, sometimes de degradation products can be more harmful and toxic to the environment that the original pollutant. As an example, Sulfanilic Acid (SA) and 1-Amino-2-Naphthol (1A2N) which are the degradation products of the azo dye Acid Orange 7 (AO7), presents a recalcitrant character and are potentially genotoxic to humans, fish, and animals (Pan et al. 2011); suggesting a combination of the technologies as a reasonable scheme for treating wastewaters containing EPs.

Hence, in view of the widespread detection of AO7, nitrates, 2,4-D, CB and IBP in the environment and the limited data available in the literature or the absence of efficient technologies on the treatment of these priority pollutants in contaminated water, the main objective of the present work is to propose the use of up-flow stirred packed-bed reactor (USPBR) for the degradation of the selected EPs under anaerobic conditions. Additionally, to combine this process with new redox mediators is an innovative approach for the studied micropollutants removal, as well as, the development of a post-treatment for degradation by-products.

1.2. Contamination levels and toxicity effects on human health and environment of the studied pollutants

1.2.1. Acid Orange 7

Despite being a well-studied azo dye, there is no literature review about the contamination levels of Acid Orange 7 (AO7) in water and wastewaters. Only general information about the azo dyes disposal percentage can be found, as

an example, Konsowa and coworkers (Konsowa et al. 2011) reported that at least 4 % of the azo dye production is going to domestic and industrial wastewater every year. Other research establish that over 15 % of the textile dyes are missed into the wastewater streams (Park & Choi 2003). Furthermore, the effluents from textile industries are very coloured, which is the maximum indicator of water contamination (Garg & Tripathi 2013).

The inadequate azo dyes wastewater disposal in aquatic ecosystems affects the entering of sunlight in the water bodies and the oxygen transfer, provoking the death and putrefaction of the aquatic fauna (Bafana et al. 2009; Solís et al. 2012; Sahoo & Gupta 2005). For the human health it is greatly toxic, due to its ingestion can produce eye, skin, mucous membrane and respiratory tract irritation. It can also cause nausea, dermatitis, loss of bone marrow, acute headache and tumours (Gupta et al. 2006).

1.2.2. Nitrate

The increase of nitrates concentrations in marine ecosystems, ground and surface drinking waters is of concern for research community, due to they are degrading the quality of the water on a worldwide scale, and have been converted a prevalent environmental problem (Smith 2003). Currently, nitrates concentrations around 1 mgNO_3^--N/L signify that is provoke by human activity and concentration levels above 3 mgNO_3^--N/L is considered water contamination (USEPA 2016a). For example, in ground waters nitrates concentrations could overcome values of 100 mgNO_3^--N/L and in surface waters of 25 mgNO_3^--N/L, whilst for aquaculture systems and marine aquaria

nitrate concentrations values might be near to 500 mgNO_3^--N/L (Camargo et al. 2005). Thus, the Environment European Agency (EEA) regulations and the United States Environmental Protection Agency (USEPA) have established specific global rules to address this problem, by ruling drinking water should not contain more than 11,3 mgNO_3^--N/L and 10 mgNO_3^--N/L, respectively (McAdam & Judd 2006; USEPA 2009).

The high nitrates levels can have harmful effects for human health and animal life; the principal contact with this pollutant can be through the ingestion of contaminated drinking water and/or eating foods, such as vegetables and meat. For instance, in the aquatic environment an increase in nitrates concentration in the water can induce to an increment in the algae growths, which produces sicknesses in fish and even the death in large numbers of species, due to they can decrease considerably or eliminate the oxygen present in water (USEPA 2016b). In animals, such as sheep and cows the nitrates can cause an equal sickness to methemoglobinemia and the principal symptoms are: trouble breathing, absence of coordination, blue colouring of mucous membrane, abdominal pain and vomiting (Goldberg 1989). For humans, the ingestion of nitrates can provoke methemoglobinemia and play a potential role in the development of cancers of the digestive tract, and in the etiology of insulin-dependent diabetes mellitus. Other scientific researchers suggest that its ingestion can also cause, coronary heart disease, cancer in bladder and ovarian, or cause spontaneous abortions (Camargo & Alonso 2006).

1.2.3. 2,4-dichlorophenoxyacetic acid

2,4-dichlorophenoxyacetic acid (2,4-D) is a badly biodegradable contaminant, due to it can only be mineralized in fresh water by microorganisms at concentrations under 10 µg/L and cannot be almost decomposed to concentrations above than 1 mg/L (Brillas 2000). In treated and untreated water supplies, 2,4-D detection limits have been found between 1,1-2400 µg/L (Jervais et al. 2008). A study made with the activated sludge respiration test reveals that 2,4-D presents a toxicity value of 213 mg/L (Sanchis et al. 2013). Hence, the USEPA has established an enforceable regulation for 2,4-D, setting a maximum contaminant level (MCL) of 0,07 mg L^{-1} based on its toxicity category (I); the USEPA toxicity class ranges from I-III, being I the most toxic (USEPA 2009).

The studied pesticide is regarded of moderate toxicity for animals, however, dogs which have been exposed to 2,4-D have shown the occurrence of a malignant lymphoma (Hayes et al. 1991). Nevertheless, the humans exposure may have many risks for human health, such as, coughing, burning, dizziness, loss of muscle coordination, nausea, diarrhea and vomiting (USEPA 2007). In addition, the 2,4-D provokes damages in the nervous system, including, loss of coordination, inability to walk, rigidity in the arms and legs, inflamed nerve endings, fatigue, stupor, coma, and death (USEPA 2005). Continuous application may cause soil percolation, groundwater contamination and the exposure of agricultural workers for extended periods of time, can produce serious skin and eye irritation (Badellino et al. 2007; Fontmorin et al. 2012).

1.2.4. Chlorobenzene

The chlorobenzene (CB) concentrations levels in surface waters are usually in the range of ng/L to µg/L and maximum concentrations of 0,2 mg/L can be achieved in areas near to industry sources. Although, CB concentration levels in industrial wastewaters can be high and vary depending to the nature of the utilized process (Malcolm et al. 2004). Van Wijk and coworkers (Van Wijk et al. 2004) made a hazard evaluation of CB to the marine environment, obtaining a risk of environmental concentration of 32 µg/l in the studied aquatic marine environment. Accordingly, the USEPA has established a MCL for CB of 100 µg L^{-1} (Burns et al. 2013).

Its production, use and inadequate removal leads with their presence in groundwater, surface water and soil samples, implying serious threats to the public health and animals (Chary et al., 2012; Field and Sierra-Alvarez, 2007; Ma et al., 2012). A severe inhalation exposure of animals to CB causes muscle spasms, tremors, narcosis and restlessness (USEPA 2000). For humans, the contact with CB can irritate the skin, eyes, nose and throat; the exposure to broad amounts of CB can also provoke negative nervous system effects, including unconsciousness. Workers breathing vast quantities of CB can feel headaches, muscle spasms, and undesirable effects on the bone marrow. Other human health impacts related with repeat exposure to small amounts of CB over long periods of time are not known (USEPA 1995).

1.2.5. Ibuprofen

Ibuprofen (IBP) is present in the environment generally at trace levels (ng/L to µg/L), but this amount can be enough to cause toxic effects (Hernando et al. 2006). For example, in river waters has been detected a concentration of IBP below 10 µg/L (Vieno et al. 2005; Brun et al. 2006). Different research were carried out in United States and Europe in order to determine the presence and concentration of IBP in streams and sewage effluents; such studies revealed that around 9,5 % of the examined streams in the United States had traces of IBP with a minimum concentration of around 0,018 µg/L and a maximum of about 1,0 µg/L. Whilst in Europe the sewage effluent presented a mean concentration of 0,22 µg/L (Kolpin et al. 2002; Heberer 2002). Rodil et. al (Rodil et al. 2012) performed a monitoring programme on sewage, surface and drinking water on Galicia, Spain; the research showed an average value of IBP higher than 0,1 µg/L in sewage, while in surface and drinking water the levels of IBP are below 10 ng/L.

The aquatic environment is most at risk, because of the presence of IBP; for instance, IBP has been shown to inhibit the growth of duckweed *Lemna minor* at various concentrations ranging from 1 µg/L to 1 mg/L, with the strongest effect observed at 1 mg/L (Pomati et al. 2004). IBP also affects the reproduction of crustacean *Daphnia magna* at a minimum concentration of 13,4 mg/L (Heckmann et al. 2007). The growth of some bacterial and fungal species have been affected by the traces of IBP present in waters, as well as, genotoxic effects for fish and cytogenetic effects in freshwater bivalves (Ragugnetti et al.

2011; Pomati et al. 2006; Parolini et al. 2011). However, in terms for human health dangers exists knowledge gaps.

1.3. Alternatives for wastewater treatments

The fresh water is an essential component for the evolution of the world economy, but is a scarce natural medium to the aquatic environment and a key factor receiving the waste discharges (Lomborg 2001). In the first half of the twentieth century, initial efforts for water pollution control prevented that human wastes contaminate the water sources; however, the fast population growth and industrial increase demands a huge quantities of natural resources, altering the situation dramatically (USEPA 2004). On the other hand, water streams around the world have faced the anthropogenic contamination, due to principally the untreated wastewater or the inefficiently clean effluents of the WWTPs (Spänhoff et al. 2007). Thus, the sustainability of the modern life is based on a proper employ of the existing water sources and the recovery of the wastewaters effluents.

The conventional technologies for treating wastewaters have shown to be ineffective to handle with contaminated aqueous effluents, due to principally the refractoriness of numerous organic compounds (Hancock 1999). Therefore, the major concern has been the development of new technologies that allow reducing the impact of such wastewaters, before to be discharge into the environment. The available technologies are classified in three basic processes: physical, chemical and biological treatments; the election of a particular method depends of the type of pollutants, the volumetric flow rate to be treated and the

organic load. Each technology presents its own benefits and limitations. Table 1.1 briefly outline the basic wastewater treatment technologies, as well as, its main advantages and disadvantages.

Table 1.1.Basic wastewater treatment technologies, benefits and limitations

Treatment	Technologies	Benefits	Limitations	References
Physical	Sedimentation, flotation, coagulation, adsorption and membranes.	-Simple and low cost processes. -Reduce the turbidity of wastewater. - They are applicable for treating small or huge volumes of water.	They are not capable of to lower the organic load and the toxicity of the waste effluents to the established levels.	(Liu et al. 2009; Forgacs et al. 2004)
Chemical	Oxidative processes, ion exchange, reverse osmosis, chemical precipitation and electrochemical processes.	- Relatively tolerant to toxic pollutants and temperature changes. - The biodegradation process proceeds rapidly and is quite predictable. - They are more amenable for the automatic control.	- Employ high quantities of chemical additives. - High operational costs.	(Robinson et al. 2001; Samer 2015; Semerjian & Ayoub 2003)
Biological	Aerobic and anaerobic processes.	- It is the cheapest technology. - They are capable of accomplish high organic removal efficiency. - Low energy consumption.	- The biodegradation processes are pretty slow. - Presents problems with the bulk and rising sludge.	(Wiszniowski et al. 2006; Ros & Zupančič 2004; Ahn et al. 1999)

As we can see in the summary table, indubitably biological processes seem to be the most inexpensive and environmentally suitable treatment (Girardi et al., 2013; González et al., 2006; Liang et al., 2013). Generally, the aerobic processes are more adequate for treating low organic loads, whilst anaerobic processes are appropiate to deal with high organic loads (Chan et al. 2009). On the other hand, anaerobic degradation involves various species of microorganisms whilst the aerobic degradation is principally a single species phenomenon. Moreover, in anaerobic process less sludge is generated and therefore, the sludge handling costs are reduce. Anaerobic treatments also present lower energy requeriments, due to the fact that aeration is not necessary and methane would be produced as by-product, which could convert the anaerobic wastewater treatment in a net energy producer (Marchaim 1992).

The only serious disadvantage of the traditional anaerobic biological systems is the requirement of long hydraulic residence times (HRT). As an alternative, biofilm systems within fixed-bed or fluidized-bed reactor have been exploited for pollutants degradation. Although, effective for several degradation process, the use of fluidized-bed for toxic pollutants removal could be substantially costly, as a result of the greater energy needed to suspend the attached biomass in the reactor. Therefore, fixed-bed reactors have appreciable potential to comply the above requirements.

1.4. Biofilm and packed-bed reactors

The bio-films have been successfully utilized in wastewater treatments for more than a century; its main advantage is the positively influence of the solid surfaces on bacterial activity. The biofilm activity is not proportional to the amount of attached biomass, but raises with the thickness of the biofilm until certain level; thus, over this level the diffusion of the nutrients converts in a constraining factor. Therefore, a stable, thin and active biofilm offers many benefits in water and wastewater processes (Lazarova & Manem 1995). Additionally, it has been proven that fixed cultures are more resistant to changes in environmental conditions (such as, pH, temperature, toxic substances, nutrient concentrations and metabolic products), than suspended cultures (Wingender & Flemming 2011).

Moreover, biofilm systems utilize support media with a high surface area to maintain the biomass concentrations up to 10 times more than the habitual concentration used in conventional active sludge process (Ong et al. 2000). The use of high biomass concentration enables the biofilm systems attain a great volumetric removal capacity and the profit to have a more compact system (Lee et al. 2004). In addition, biodegradation is facilitated by enhanced gene transfer between biofilm organisms and increased bioavailability of pollutants as an outcome of bacterial chemotaxis (Singh et al. 2006).

The packed-bed biofilm reactor, immobilizes the culture over the surface of the packing medium, reducing drastically the loss of biomass in the effluent. In this type of reactor the biofilm is not damaged for a small quantity of oxygen and are

more stable (Liu & Pfeffer 1989). Comparing fixed-bed reactors against fluidized-bed reators, a several advantages can be noticed; for example, presents higher amounts of biomass concentrations, enabling higher loading rates. They are more resistent to shock loading of toxic pollutants, therefore, require shorter time to return to normal functioning (Dey & Mukherjee 2010). Another important benefits are the small reactor size, high removal efficiency, reduce the bad oddors and sturdiness to hydrodynamic changes. Besides, the fixed-bed reactors do not present problems to separate the catalyst from the reactor effluent; which constitutes another disadvantage for diverse fluidized-bed systems, because the catalyst recovery can be pretty difficult and needed considerable equipment costs (Hill 1977). On the other hand, packed-bed reactors are usually operated at a constant flowrate of the feeding solution at a established concentration. The operation conditions are frequently selected in a very conservatively manner, with a view to not violate the quality demands in the outlet of the reactor (Benthack et al. 2001). Additionally, in packed-bed reactors the fluid can flow like up-flow or down flow mode, in single or multiple pass (with recycling) and with fluids moving axially to the bed height in concurrent or countercurrent flow (Gómez-De Jesús et al. 2009).

For a fixed-film in packed-bed reactors an appropriate type of packing material should be selected; it have to meet the following requirements: availability of high amounts of the material, be a low cost material, and mechanically and microbiologically stable (Jördening & Buchholz 1999). A thorough description of different carbon materials are presented below.

1.5. Carbon and sewage slugde based materials as catalysts supports

The catalytic processes represent more than 90 % of the chemical production in use all over the world. The role of the catalysts consists of increase the rate and control the selectivity of the involved chemical reactions. The carbon materials have been utilized for ages in the heterogeneous catalysis, due to they may act as direct catalysis and satisfy most of the desirable properties required for an appropiate support; between these properties it can be found inertness, stability under reaction and regeneration conditions, adequate mechanical properties, several porous structure or the possibility to control the surface chemistry, and their cost is generally lower than the conventional supports (Rodríguez-Reinoso 1998).

1.5.1. Activated carbon

The activated carbon (AC) is well known like a greatly efficient adsorbent, as a result of its large surface area (up to 3000 m^2 g^{-1}), adjustable surface chemistry characteristics and great degree of surface reactivity (Dias et al. 2007); which converts it in a pretty versatile material. A peculiar benefit of the AC is the possibility of adapting its physical and chemical features for the purpose of optimize its effectiveness for accurate applications; meaning that both its pore structure and surface chemistry can be changed to meet the demands of the considered catalytic reaction (Pereira et al. 2004). The starting material and the activation method utilized for AC production define the surface functional groups; thus, the activation can refine the pore structure, and as result, the meso, micro and ultra micropores are formed, producing high surface areas

(Mohan & Pittman 2006). Moreover, AC has been proven to be an effective inert porous carrier material, being capable of distributing chemicals on its large hydrophobic internal surface, thus making them accessible to reactants (Yin et al. 2007); the AC is fairly inert, especially in the absence of oxygen, and exhibits an adequate mechanical resistance (Fuente et al. 2001).

The AC has been used in a wide range of applications, such as adsorbent, catalysts and catalysts supports, for the removal of contaminants from liquid or gaseous phases, and recovery or purification of chemicals (Derbyshire et al. 2000; Anisuzzaman et al. 2015; Ban et al. 1998).

1.5.2. Sewage sludge based materials

The sewage sludge is an inevitable secondary product from the wastewater treatments and remains in high quantities all over the world; for example, in the European countries, the yearly production of dry solid sludge is more than 10 million tons (Laturnus et al. 2007). Consequently, these high quantities of waste materials are very difficult to eliminate and therefore, they are available practically at a free cost in order to be utilized for several applications (Fytili & Zabaniotou 2008); generally, the waste materials is reuse in compost manufacture, agriculture, biogas production, etc. Nowadays, the utilization of solid wastes for the production of low cost adsorbents has obtained a great interest in the research community, due to it can be solved two main environmental problems: firstly the amount of waste materials will be reduced considerably and secondly, the new low cost adsorbent may decrease the pollutants in wastewaters in a cost effective manner (Athalathil et al. 2014).

Sewage sludge materials are also potentially adequate for the production of activated carbon due to its carbonaceous structure and substantial content of organic matter. The preparation of these carbon materials has been optimized, obtaining surfaces areas up to 900 $m^2 g^{-1}$ after chemical activation (Ocampo-Pérez et al. 2012). Furthermore, several authors have studied the use of sewage sludge based materials for various purposes and for diverse pollutants (Wen et al. 2011; Gu et al. 2013; Graham et al. 2001; Rozada et al. 2003).

1.5.3. Carbon nanotubes

Carbon nanotubes (CNTs) are seamless cylinder-shaped macromolecules, with a small radius in the range of a few nanometres and a length up to several micrometres (Ong et al. 2010). They have been chosen to be a highly promising material for catalytic appliances, due to their extraordinary mechanical properties, sole electrical properties, high chemical and thermal stability, and a huge specific surface area (Serp et al. 2003; Rao et al. 2007). In addition, CNTs also present small, hollow and layered structures, which make them very promising adsorbents for many organic contaminants; other important characteristics are their fibrous shape and well developed mesopores that contribute to superior removal capacities. Otherwise, CNTs can be chemically modified, increasing in this way their adsorption capacity, and can conduct electrons (Yu et al. 2005; Gupta et al. 2011).

CNTs present an excellent sorption capacity and high sorption efficiency when are compared with granular and powder activated carbon, because of their high surface active site to volume ratio and controlled pore size distribution. Broadly

research studies have found that the adsorption capacity of CNTs depends of the surface functional groups and the nature of the sorbate (Ong et al. 2010). Hence, they have been starting to gain a special interest for the treatment of water and wastewater (Gong et al. 2009; Cho et al. 2011; Yu et al. 2014).

1.5.4. Carbon xerogels

Carbon xerogels (CXs) are new porous carbon materials, that are prepared of the sol–gel polycondensation of some monomers (resorcinol and formaldehyde) and a subsequent carbonization of the organic xerogels (Pekala 1989). CXs have exceptional characteristics, for example, high surface area (400–1200 m^2 g^{-1}), great porosity, manageable pore size, high density (0,6–0,8 g cm^{-3}) and conductivity (Samant et al. 2004; Job et al. 2006). They can be prepared in different form such as, monoliths, thin film or powder; their textural and structural properties can be controlled in accordance with the synthesis and processing conditions, as an example, carbonization temperature, pH of preparation and drying conditions. The major benefit of CXs is the possibility to adapt their properties for specific applications (ElKhatat & Al-Muhtaseb 2011; Job et al. 2005; Job et al. 2004). Thus, CXs are utilize in a numerous applications (Figueiredo et al. 2011; Apolinário et al. 2008; Girgis et al. 2011).

1.6. Coupling anaerobic-aerobic treatments

The couple anaerobic-aerobic systems have been notably used in municipal and industrial wastewater treatments during years. In recent ages, the high rate anaerobic-aerobic bioreactors have been more used than the conventional

anaerobic-aerobic treatment plants, for treating wastewaters with high chemical oxygen demand (COD) (Chan et al. 2009). Even though, in the anaerobic treatments high removal efficiencies are obtained, the generated effluent contains solubilized organic matter, because of the high inorganic strength of the wastewater; which is convenient for the aerobic treatments. For this reason, a post-treatment with aerobic treatment is necessary to comply with the effluent disposal regulations (Gray 2005). Developing novel technologies which connect anaerobic and aerobic ways of treatment will serve to strenghthen such treatments, with the purpose of eliminate the biorefractory pollutants to the point wherein can be reused as potable water.

The use of membrane bioreactors (MBRs) for wastewater treatments have acquired a growing attention, due to it takes advantage of the rapid development in membrane manufacturing and has the potential to fundamentally advance the biological treatment process. MBRs posses different benefits, such as excellent effluent quality, a high biomass concentration without concern for sludge settling problems, a simple flow configuration and small footprint (Le-Clech et al. 2006). The MBRs are also capable of retain organic contaminants with small molecular weight compared with conventional activated sludge process; MBRs with ultra-filtration membrane are able to retain several species of viruses (Neoh et al. 2016). The MBRs contains two main parts: the biological unit for the biodegradation of waste pollutants and the membrane module for the physical separation of the treated water; MBRs can be classified in two main groups, taking into account their configuration. The first group, comprises outer membranes that are inside of the bioreactor and the

second group, includes the recirculation of the mixed liquor across the membrane module, which is outside of the bioreactor (Cicek 2002).

In this view, the combination of an anaerobic biological process with a physical filtration is regarded that are the best economical and viable solution for the completely degradation of recalcitrant pollutants.

Chapter 2

Hypothesis and objectives

2.1. Antecedents

It has become evident that conventional treatment plants are not able to eliminate the wide range of recalcitrant organic compounds that are present in wastewaters and far less the by-products that can be generated; which can present higher environmental problems and health problems than the original ones. Owing to its capacity to removal high organic content, shorter HRT, smaller reactor volumes and achieve low amounts of sludge and suspended solid, the anaerobic up-flow packed-bed reactor has shown to be the better option for treating several of these toxic pollutants (Kocadagistan et al. 2005).

The removal of nitrates, 2,4-D, CB and IBP from wastewater, using anaerobic up-flow packed-bed reactor has been scarcely researched in the literature; likewise, the aerobic post-treatment of the refractory by-products of AO7, employing membrane filtration processes. Therefore, in the last decade new possibilities for the use of membrane modules integrated to an aerobic bioreactor have been found to be favourable for water and wastewater treatment. The integrated system, well-known as MBR, may be particularly convenient for reuse and recycling wastewater, due to its high quality and disinfected effluents (Cicek et al. 1998).

It is worth nothing that in anaerobic packed-bed reactors the support material should play various important roles, such as, be an excellent support for the attachment of microorganisms and growth of a biofilm, act as redox mediator by transferring electrons from the carbon source, and the synergetic behaviour of

the biological carbon systems has to exhibit higher efficiencies than expected for the biodegradation or adsorption alone. For this reason, an appropriate type of packing material should be selected and the improvement of the state of the surface, pore size and the geometries of the support carbon materials can enhance the efficiency of the anaerobic packed-bed reactors (Córdoba & Siñeriz 1990; Young & Dahab 1983).

In this context, the present work is for the study of the biodegradation of different pollutants of concern (nitrates, 2,4-D, CB and IBP) in an anaerobic up-flow stirred packed-bed reactor and the subsequent degradation of the breakdown products of the AO7 in an aerobic membrane reactor system; the investigation of different carbon materials as catalytic support is a novel approach for anaerobic packed-bed reactors.

2.2. Hypothesis

On one hand, the anaerobic up-flow stirred packed-bed reactor capable of reducing azo dyes also will be able of degrading emerging pollutants and nitrates, and its integration with an aerobic membrane post-treatment will degrade the by-products of AO7 reduction. Its implementation in optimal conditions might suppose the reused of the treated wastewater as potable water.

On another hand, the utilization of novel mesoporous materials is supposed to present technological benefits, principally for the degradation of major molecules and speed up the process.

2.3. Objectives

The general objective of the present work is to investigate the degradation of nitrates, 2,4-D, CB and IBP in an anaerobic up-flow stirred packed-bed reactor. The preparation, surface chemistry modification and characterization of different new catalytic support materials were carried out, in order to study the influence of the surface chemistry and textural properties of the carbon materials on the catalytic yield. As well as, the design of an aerobic membrane unit for the subsequent degradation of AO7 breakdown products, offers an advantageous coupled system for treating high strength wastewaters.

The following specific objectives were proposed to accomplish the main objective:

- To compare the anaerobic nitrate removal of an agitated packed-bed reactor filled with biological sludge carbonaceous material with an agitated packed-bed reactor filled with biological activated carbon. To study the significance of applying a special agitation to the biological beds. To determine the maximum nitrate concentration degraded in the bioreactors systems. Additionally, to describe the denitrification process in both reactors systems with the Michaelis-Menten kinetic model **(Chapter 4)**.

- To investigate the adsorption capacity of the activated carbon to CB, 2,4-D and IBP. To study the biodegradation of the selected pollutants in a continuous anaerobic agitated packed-bed reactor filled with biological activated carbon. To determine if the Michaelis-Menten kinetic model describe adequately the degradation process for the target contaminants **(Chapter 5)**.

- To characterize chemically and texturally the novel carbon materials **(Chapter 6)**.

- To describe a sequential anaerobic-aerobic process for complete mineralization of AO7. To investigate the influence of periodical agitation of the biological bed on decolourization rates in the anaerobic system working in continuous. To determine the effect of HRT on COD, colour and aromatic removals in the aerobic stage. To study the total aromatic amine removal in anaerobic/aerobic sequential system in an operation period **(Chapter 7)**.

Chapter 3.

Methodology

3.1. Model contaminants

The selected model contaminants were potassium nitrate (KNO_3, 99,4% purity), ibuprofen sodium salt (IBP; 100% purity), chlorobenzene (CB; 99,99% purity), azo dye Orange II (C.I. Acid Orange 7, 99% purity), all of them purchased from Sigma-Aldrich, and 2,4-dichlorophenoxyacetic acid (2,4-D, 99% purity), obtained from Acros Organics. Chlorobenzene was dissolved in ethanol (99,99% purity, Aldrich) to make a standard stock solution of 0,1 M. The chemicals and reagents in the investigation were received in analytical grade; the chemical structure of the emergent pollutants is shown in figure 3.1. All the solutions were prepared with deionised water.

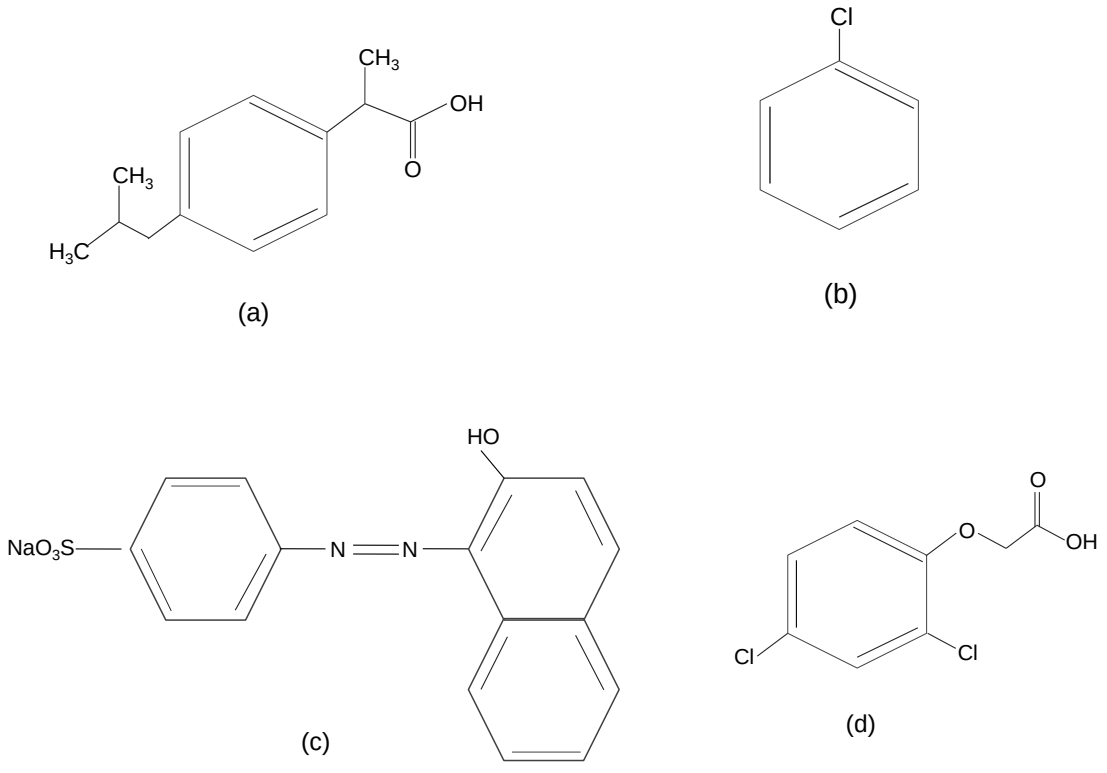

Figure 3.1. Chemical structure of (a) IBP, (b) CB, (c) AO7 and (d) 2,4-D.

3.2. Procedures

3.2.1. Anaerobic up-flow stirred packed-bed reactor (USPBR)

3.2.1.1. General chemicals and materials

Sodium acetate (CH_3COONa, 99% purity) was purchased from Sigma-Aldrich and was provided as carbon source for the microorganisms. Commercial AC (Merck, granules of 2,5mm) was used as support material in USPBR; it was crushed and grains of 0,3–0,7 mm were separated. Carborundum granules was obtained from Carlo Erba Reagents and utilized as inert solid diluent.

3.2.1.2. General experimental set-up

The USPBR system and operating parameters used in this study are similar to those described by (Mezohegyi et al. 2008). It consists of a cylinder with a diameter of 15 mm and a useful volume of 10 ml. It was filled with the mixture of 10 g of carborundum granules and 1 g of commercial AC with the size of 25–50 mesh. To prevent washing out of AC, two filters were placed into the top and bottom of the reactor. The USPBRs were working at a constant temperature of 35 ± 1°C. The anaerobic conditions in the feeding bottle were maintained by both cooling of the solution at 5 °C and bubbling of an inert gas. The redox potential was continuously measured where the outlet immediately left the USPBRs, and remained approximately below –500 mV (referred to Ag+/AgCl electrode). The reactors were built together with an agitation system that makes possible to apply a very fine and slow stirring (1rph) in the BAC bed, to avert outlet and loss of biomass (Figure 3.2).

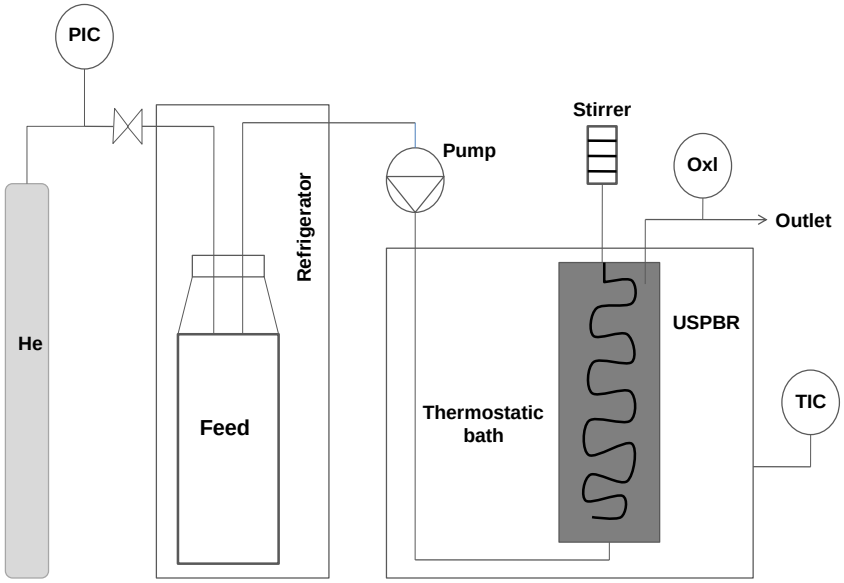

Figure 3.2. Anaerobic up-flow stirred packed-bed reactor system.

To prepare the biological system, anaerobic sludge with mixed culture was obtained from the wastewater treatment plant from Reus, Tarragona, Spain. The sludge was filtered by a micro filter with a pore size of 20–25 μm to only have single cells and/or spores. The mixed cultures were adapted to grow with each studied pollutants, sodium acetate and the basal media. To prepare the biological system, the mixed cultures were pumped through the AC for a week; under anaerobic conditions at 35 ± 1ºC. During this period the biofilm grew on the AC surface resulting in the so-called biological activated carbon (BAC).

3.2.1.2.1. Specifications for nitrate biodegradation

In Chapter 4, a set of two reactors was prepared to carry out the study and they were started up in parallel. The same experimental procedure, described in session 3.2.1.2, was followed for assembling both bioreactors; but, one was

filled with sludge carbonaceous materials (SCM) prepared by (Athalathil et al. 2014) and the other with the AC. The SCM follows the same specifications of the AC to be used as catalysts. In this study was used helium to maintain the anaerobic conditions.

During the first period of operation, the entering feed was 50 mgNO_3^-/L solution containing CH3COO$^-$/$NO_3^-$with a mass ratio of 2:1 (in order to avoid the carbon-limiting condition) and the basal media with microelements (1 ml of each basal media per litre of feeding solution). In this chapter the basal media contained the following composites (mg L^{-1}): $MnSO_4 \cdot H_2O$ (0,155); $CuSO_4 \cdot 5H_2O$ (0,285); $ZnSO_4 \cdot 7H_2O$ (0,46); $CoCl_2 \cdot 6H_2O$ (0,26); $(NH_4)6Mo_7O_{24}$ (0,285); $MgSO_4 \cdot 7H_2O$ (15,2); $CaCl_2$ (13,48); $FeCl_3 \cdot 6H_2O$ (29,06); NH_4Cl (190,9); KH_2PO_4(8,5); $Na_2HPO_4 \cdot 2H_2O$ (33,4); K_2HPO_4 (21,75); these chemicals were obtained from Sigma-Aldrich.

In addition, for the biological system an identical treatment as the one specified in session 3.2.1.2 was followed, to prepare and adapt the mixed culture over the SCM.

The effect of different nitrate concentrations (25–700 mgNO_3^-/L) on the denitrification rate and nitrate removal was examined. The flow rate of the feed was varied between 25 and 350 ml h^{-1} and was ensured by a peristaltic pump.

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3.2.1.2.2. Specifications for CB, 2,4-D and IBP biodegradation

In Chapter 5, the entering feed was 25 mg L^{-1} solution for CB and 2,4-D, and 10 mg L^{-1} for IBP; it contain also 100 mg L^{-1} of sodium acetate as substrate and the basal media with microelements. In these serial of experiments the basal media contained the following compounds (mg L^{-1}): $MnSO_4 \cdot H_2O$ (0,155), $CuSO_4 \cdot 5H_2O$ (0,285), $ZnSO_4 \cdot 7H_2O$ (0,46), $CoSO_4 \cdot 7H_2O$ (0,307), $(NH_4)6Mo_7O_{24}$ (0,285), $MgSO_4 \cdot 7H_2O$ (15,2), $Ca(NO_3)_2$ (19,93), $Fe_2(SO_4)_3$ (21,47), KH_2PO_4 (8,5), $Na_2HPO_4 \cdot 2H_2O$ (33,4), K_2HPO_4 (21,75). These chemicals were obtained from Sigma-Aldrich. As we can see, many of the compounds used in the basal media mentioned in session 3.2.1.2.1 were substituted by the above-mentioned, due to, that much of these composites contains chlorine and taking into account that the studied pollutants present this composite in their structure, we decided to substitute them.

On the other hand, nitrogen (N_2) was used to maintain the anaerobic conditions; therefore, a gas chamber was placed before the entrance to the USPBR system and at the same level of the bioreactor, in order to capture the excess of N_2 contained in the feeding solution. The flow rate of the feed was varied between 0,8 and 25 ml h^{-1} for CB and 2,4-D, and between 1 and 25 ml h^{-1} for IBP; the flow rate was insured by a micro pump (Bio-chem Valve Inc., ref. 120SP2420-4TV). Besides, to prepare the biological system for IBP, CB and 2,4-D the culture was fed with 10, 25 and 100 mg L^{-1} of the target micropollutants solution, respectively.

3.2.2. Adsorption experiments

The adsorption experiments were carried out only for CB, 2,4-D and IBP on the commercial AC and was investigated under batch conditions; the sorbate/sorbent suspensions contained 100 ml of the target solution. Experiments were executed on individual component solutions at different concentrations (IBP: 3 - 30 mg L^{-1} and 2,4-D: 50 – 250 mg L^{-1}); 0,1 g and 0,2g of AC were used, respectively. For CB initial concentration of 300 mg L^{-1} was mixed with different weights of AC (0,02 – 0,6 g) and the adsorption tests were accomplished in amber bottles. In parallel, one blank control (CB solution without AC) was included in order to evaluate the evaporative losses. The temperature and shaking remained constant during each experiment at 35ºC and 200 rpm. Aliquots were taken at different time intervals until equilibrium was attain.

3.2.3. Preparation and surface chemistry modification of novel catalysts

The catalysts preparation, surface chemistry modification and characterization were fulfilled during a research stay at the Faculdade de Engenharia da Universidade do Porto in the Laboratório de Catálise e Materiais research unit.

3.2.3.1. Chemicals

Nitric acid (≥65% purity), hydrochloric acid (≥37% purity), resorcinol (99% purity) and formaldehyde solution (37% purity) were purchased from Sigma-

Aldrich. Melamine (≥99% purity) and urea (99,5% purity) were obtained from Fluka and Acros Organics, respectively.

3.2.3.2. Carbon materials

The first type of carbon material prepared was a commercial NORIT GAC 1240 Plus M-2016 activated carbon, which was previously crushed and separated in granules of 25-50 mesh size; being this initial material denoted as AC.

Secondly, a commercial MWCNT sample (Nanocyl-3100) was prepared; it has an average diameter of 9,5 nm, average length of 1,5 µm and carbon purity higher than 95%.

Finally, a set of carbon xerogel (CX) at different initial pH (5,3 and 6,9) were prepared in parallel.

3.2.3.3. Preparation of carbon xerogels

The following procedure is similar to that described by (Messele 2014). 25 g of resorcinol were mixed with 40 ml of distilled water under magnetic stirring until was completely dissolved. Then, the pH was adjusted to 5,3 and 6,9 adding a few drops of NaOH 2M solution. Later, 34 ml of formaldehyde solution were added under stirring and the final pH was adjusted to 5,3 and 6,9 by addition of HCl 0,1M solution. The gelation was achieved in a water bath at 85 ºC during three days. After this period, the gel was crushed and dried in an oven for 4

days; the temperature in the oven was increased as follows: 1st day at 60 ºC, 2nd day at 80 ºC, 3rd day at 100 ºC and last day at 120 ºC. The dried gel was carbonized under N_2 flow (100 ml min^{-1}) at 600 ºC and the carbonization protocol was carried out as follows: (1) from room temperature to 200 ºC and hold for 2h, (2) up to 300 ºC and hold for 1h, (3) up to 500 ºC and hold for 2h, (4) up to 600 ºC and hold for 2h, (5) cooling down to room temperature; the heating rate was kept constant at 2 ºC min^{-1} for all the heating program. The carbon xerogels prepared were labelled as CX followed by the respective pH value.

3.2.3.4. Chemical modification of carbon materials

3.2.3.4.1. Oxidation with nitric acid in liquid-phase

The oxidation of 7g of initial activated carbon (AC) was carried out in a 250 ml Soxhlet extraction apparatus connected to a boiling flask and a condenser. Then, 200 ml of 6 M nitric acid (HNO_3) solution was inserted in a 500 ml Pyrex round-bottom flask and heated to boiling temperature with a heating mantle; the reflux was stopped after 3 h. The oxidized AC was washed several times with distilled water until neutral pH and dried in an oven at 100 ºC during 24 h. The material was designated as sample ACO.

The commercial MWCNT was oxidized following a procedure similar to the described above. For this, 4g of MWCNTs (sample CNT) and 300 ml of 7M HNO_3 solution were introduced into a 500 ml Pyrex round-bottom flask and heated to boiling temperature with a heating mantle. The boiling flask was

connected to a condenser and the reflux was stopped after 3 h. The oxidized sample (CNTO) was wash extensively with distilled water to neutral pH and dried in an oven at 100 ºC during 24 h.

3.2.3.4.2. Treatment with urea and melamine

Different sets of catalysts were functionalized with nitrogen containing compounds, such as urea and melamine. The starting materials and the proceeding used in each case are described below.

3.2.3.4.2.1. Activated carbon and oxidized activated carbon

For samples AC and ACO, 2 g (of each material) were mixed with 100 ml of 1M urea aqueous solution and stirred at room temperature during 24 h. After this time, the materials were filtered and dried in the oven at 100 ºC for 24 h. The treated samples were carbonized under N_2 flow (100 ml min^{-1}) at a heating rate of 10 ºC min^{-1}up to 600 ºC and kept at this temperature for 50 min. The samples were designated as AC_urea and ACO_urea.

In the case of the functionalization with melamine, 2 g of AC (or ACO) were added to a melamine suspension (1,3 g of melamine in 80 ml of ethanol, for a total volume of 100 ml) and stirred at room temperature for 24 h. The new samples were carbonized under N_2 flow (100 cm^3 min^{-1}) at a heating rate of 10 ºC min-1up to 600 ºC and held at this temperature for 50 min. The samples were identified as AC_melamine and ACO_melamine.

3.2.3.4.2.2. Carbon nanotubes

The proceeding for the impregnation of sample CNT was the same as the one described in session 3.2.3.4.2.1, but just 1 g of the catalyst was used for the functionalization with urea and melamine, and temperature was kept by 1 h. The samples were labelled as CNT_urea and CNT_melamine, in each case.

3.2.3.4.2.3. Carbon xerogels

The procedure followed in this session is similar to the described by (Messele 2014); in this case, 15 g of resorcinol and 4 g of urea (or 3 g of melamine) were mixed in 18 ml of distilled water. The solution was heated up to 90 ºC and agitated until the urea (or melamine) was dissolved. Afterward, the solution was cooled until room temperature and 20 ml of formaldehyde was added. The pH was adjusted to 5.3 and 6.9, respectively. The gelation, drying and carbonization followed the procedure stated on session 3.2.3.3. The result samples were designated as CX followed by the pH value and the nitrogen containing compound used (urea or melamine), respectively.

3.2.3.4.3. Thermal treatment

Samples ACO and CNTO were the initial materials selected to be thermally modified. The initial materials presented a broad quantity of oxygen surface groups that were removed in different types (and amounts) by applying distinct temperatures. The heat treatment of ACO and CNTO was carried out under a flow of N_2 at 100 ml min^{-1} for 1 h at 400 ºC, 600 ºC and 900 ºC, respectively; the

heating rate was 10 ºC min^{-1}. The samples were labelled as ACO (or CNTO) followed by the value of the applied temperature.

3.2.4. Sequential anaerobic-aerobic system for AO7 biodegradation

As it was mentioned in Chapter 1 and 2, the reduction of AO7 to SA and 1A2N (Figure 3.3) by sewage effluent under anaerobic conditions has been reported in the literature, but no a subsequent finishing biological treatment using aerobic MBR process; thus, a preliminary study in the complete mineralization of AO7 in a sequential anaerobic-aerobic process has been proposed to be investigated in the present research.

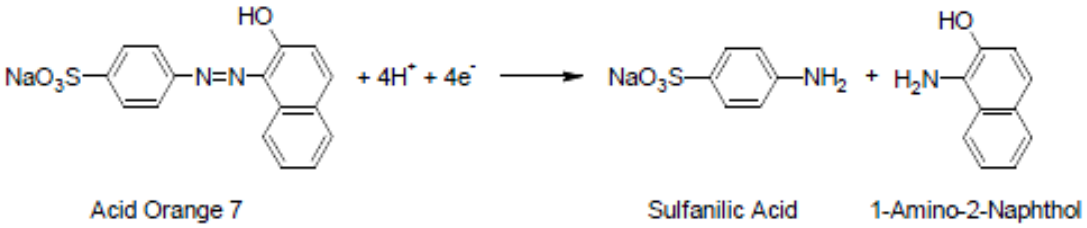

Figure 3.3. Reduction of AO7

3.2.4.1. Chemicals and materials

SA (99% purity) was purchased from Sigma-Aldrich Company, the AC and SCM were obtained as in the sessions 3.2.1.1 and 3.2.1.2.1, respectively; however, the AC was crushed and sieved into 100-200 *μ*m in size, due to is used as support material in the aerobic unit. The basal media contained the same composites as in session 3.2.1.2.1.

3.2.4.2. Experimental set-up

The laboratory scale system consists of the anaerobic USPBR and an aerobic MBR.

In the continuous anaerobic USPBR assembly and start-up was equal to the described in session 3.2.1.2, but using SCM as catalysts support. The entering feed to the anaerobic reactor was 100 mg L^{-1} AO7 solution containing the basal media with microelements and 200 mg L^{-1} sodium acetate, used as co-substrate, being both the carbon source for sludge and electron donor for azo reduction. Helium gas was continuously bubbled to maintain the anaerobic conditions in the feeding bottle. The flow rate of the feed was maintained constant at 25 ml h^{-1} and was ensured by a micro pump (Bio-chem Valve Inc., ref. 120SP2420-4TV). The outlet of the anaerobic stage was used as influent for the aerobic stage.

The aerobic MBR, as shown in Figure 3.4, consists of an aerobic sludge unit which has a diameter of 77 mm and a working volume of 500 ml, followed by a microfiltration membrane module, in order to remove the bacterial, parasites and suspended particles that are present in the sludge and in the influent solution. The microfiltration membrane used was a Nuclepore™ Track-Etched made of polycarbonate with a nominal pore size of 1µm, a diameter of 47mm and a surface area of 0,19625 m^2. The membrane module contained in the part of the effluent input an agitator, for the purpose to remove the AC that could entry with the effluent. The aerobic sludge unit is filled with 1 g of AC, used as catalytic support material. The mixed culture within the bioreactor was

maintained at room temperature 25 ± 1°C. An air pump provided continuous and good aeration through an air diffuser in the bottom of the bioreactor. The feed, containing the anaerobic effluent was introduced into the top of the aerobic sludge unit by a micro pump (Bio-chem Valve Inc., ref. 120SP2420-4TV) and the HRT was varied from 8 to 48 hours; the effluent from the aerobic unit was further flowed into the membrane module using a peristaltic pump SPETEC 12® and the final aerobic effluent of the aerobic MBR was gravity withdrawn.

The mixed culture used in the biological system was obtained by partial digestion of aerobic sludge from the wastewater treatment plant from Reus, Tarragona, Spain. The same experimental procedure, as the one described in session 3.2.1.2, was followed to prepare and adapt the mixed culture above the SCM and the AC. The anaerobic effluent was used to acclimatize the biofilm in the aerobic stage.

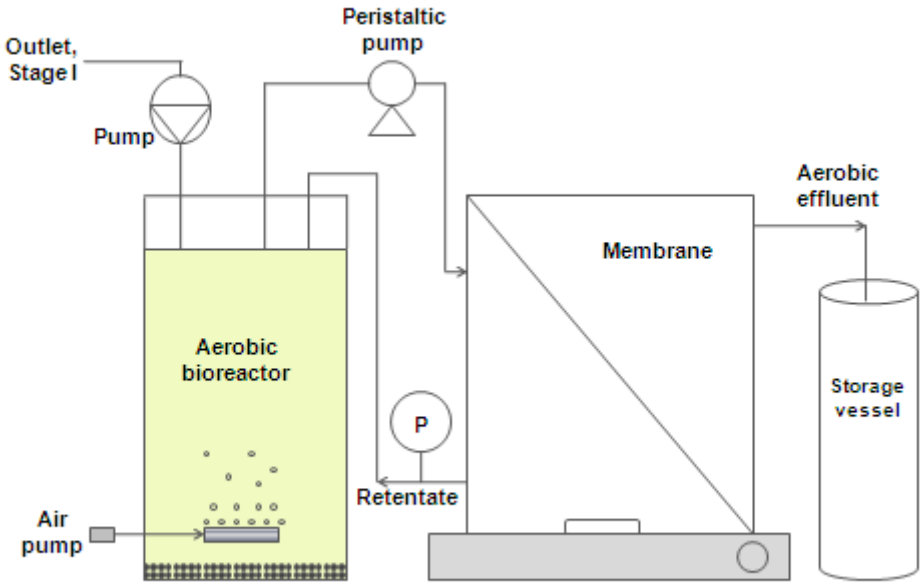

Figure 3.4. Aerobic membrane bioreactor (aerobic MBR) system.

3.3. Analytical methods

3.3.1. Measurement of 2,4-D, IBP, AO7, SA and CH_3COONa concentrations by high performance liquid chromatography

The entrance and output effluents from the adsorption studies, anaerobic and aerobic treatments were analysed using a high liquid performance chromatography (HPLC), in an Agilent Serie 1100 chromatograph using a C_{18}Hypersil ODS column (Agilent Technologies).

In the case of 2,4-D the mobile phases were methanol (99,9 % purity, Sigma-Aldrich)-acidified Milli-Q water (60:40), containing 4% acetic acid (99 % purity,

Sigma-Aldrich). The flow rate of the mobile phase was 1 ml min^{-1}, the injection volume at 20 µl and the column compartment was set at 28 ºC. The retention time (RT) for 2,4-D under these conditions was approximately 10 min. The analyses were performed at 240 nm with a diode array detector (DAD). This analytical method is based on that described by (Guo et al. 2000).

For IBP, AO7, SA and acetate the mobile phases were (99,9 % purity, Sigma-Aldrich)-acidified Milli-Q water (60:40); the Milli-Q water was acidified with $H_2SO_4$2M (pH=1,41).The flow rate of the mobile phase was set at 1 ml min^{-1}, the injection volume at 20 µl and a DAD was used to identify the compounds. IBP was determined at 190 nm (RT approximately 15 min), AO7 was determined at 487 nm (RT= 11,34 min), SA was determined at 252 nm (RT = 2,18 min) and acetate was determined at 210 nm (RT = 3,24 min). 1A2N, the other product generated during the anaerobic degradation of AO7, was not determined because of its partial precipitation.

3.3.2. Determination of CB concentration by gas chromatography

The liquid samples from the adsorption study, anaerobic and aerobic processes were analysed by means of gas chromatography (GC) in a Varian CP-3800utilizing a HP-5MS column (J&W Scientific, Folsom, CA, USA). The temperatures ramp were as follows: 40 °C for 3,0 min, heated at 20 °C min^{-1} to 100 °C, then increased at 65 °C min^{-1} to 175 °C, and finally heated at 50 °C min^{-1} to 270 °C and held for 0,5 min. The total chromatographic run time was

9,5 min and the carrier gas was helium. This analytical method is adapted from that described by (Grueiro Noche et al. 2013).

3.3.3. Measurement of nitrate, nitrite and ammonium concentrations by colorimetric methods

Samples of influent and effluent were collected every day and filtered with syringe filters with a pore size of 0.45µm. Nitrate and nitrite concentrations were determined by ultraviolet spectrophotometric screening and the colorimetric method, respectively, according to Standard Methods (APHA 1989), and using the 8500 Spectrophotometer UV-VIS (DINKO Instruments). Ammonium was analysed using a HI83099 COD and Multiparameter Photometer (Hanna Instruments).

3.3.4. Chemical Oxygen Demand

Chemical Oxygen Demand (COD) is the quantity of oxygen required to totally oxidize the organic water components in inorganic final products. COD represents a helpful measure of the quality of the water, due to it generally is used to determine indirectly the quantity of organic composites present in the water. Therefore, COD in influent and effluent samples in the aerobic MBR was determined on clear supernatants according to Standard Methods (APHA 1989).

3.3.5. Total Organic Carbon

The Total Organic Carbon (TOC) is a measure of the concentration of organic compounds in solution; having a high importance due to that TOC values show the level of mineralization in terms of CO_2 and H_2O of the samples and gives an idea of the efficiency of the process. TOC analyses were carried out in influent and effluent samples in the aerobic MBR in an Analytik Jena multi N/C 2100 TOC analyzer.

3.3.6. Characterization of carbon materials

3.3.6.1. Textural characterization

The textural characterization of the catalysts was important in case of pore size-modified carbon materials and the subsequent textural changes of the surface chemistry modifications, including the thermal treatments. This characterization was based on the N_2adsorption isotherms, determined at −196 ºC by a Quanthachrome NOVA 4200e analyser. Previous to the analysis, the samples were deaerated at 150 ºC for 5 h under vacuum. The surface area (S_{BET}) of the samples was determined by the BET method, the specific surface area of the mesopores (S_{meso}) and micropore volume (V_{micro}) were calculated by the t-method, the total pore volume (V_p) was determined when P/P_0=0,95 and the pore size (D_p) was calculated by the NLDFT method.

3.3.6.2. Chemical characterization

The determination of the pH at the point of zero charge (pH_{pzc}) was carried out similar to the procedure described by (Messele et al. 2015): 0,05 g of each sample was mixed with 50 ml of 0,01 M NaCl solution; the pH values were adjusted between 2 and 12, with the addition of 0,1 M NaOH or 0,1 M HCl solutions. The final pH was measured after 24 h of stirring at 25 ºC. Blank tests (without carbon) were also carried out to every pH, in order to eliminate the alteration on pH values due to the presence of CO_2 in the head space; the pH values measured after 24 h are considered the initial pH. The value of pH_{pzc} of each carbon material sample is the point in which the obtained pH_{final} vs. $pH_{initial}$ curve intersects the straight line $pH_{final} = pH_{initial}$.

The content of carbon, hydrogen, nitrogen, sulphur and oxygen were analysed using an EA 1108 Elemental Analyser (Carlo Erba instruments).

The X-ray photoelectron spectroscopy (XPS) analysis was accomplished in a VG Scientific ESCALAB 200A spectrometer using a non-monochromatized Mg Kα radiation (1253.6 eV); the binding energies are calibrated with respect to the C1s peak at 285.0 eV.

Chapter 4

Anaerobic nitrate removal in a novel packed-bed reactor

4.1. Introduction

There are many different technologies to remove nitrates from wastewaters as it has been mentioned in Chapter 1; each has individual advantages and disadvantages. For example, ion exchange has been developed principally to treat high concentrations of nitrate, but it is limited because there are few resins with high selectivity for nitrate and the problem of regeneration (Pintar et al. 2001; Shrimali & Singh 2001). Reverse osmosis, is capable of separating and concentrating the compounds in wastewater without making any changes to their molecular structures, but its main disadvantage is the low selectivity of the membranes used for nitrate (Ergas & Reuss 2001; Ersever et al. 2007). Membrane biotechnology is widely used for denitrification, but the presence of air can destroy the anaerobic environment and the membrane can be considerably damaged by pressure and easily contaminated (McAdam & Judd 2006; Wisniewski et al. 2001). Electro-dialysis removes nitrate from wastewater without the use of additional chemicals, but is sensitive to iron, manganese, hydrogen sulfide (H_2S), chlorine and hardness (Annouar et al. 2004; Islam & Suidan 1998). Of all of the existing techniques, biological denitrification is considered to be economically effective and feasible, and is widely used for nitrate removal (Foglar 2013).

The packed-bed reactors using biofilm are broadly utilized for nitrate removal, due to they are able to adjust the HRT necessary to carry out the biological reaction, contain high biomass concentrations reached by fixing them over diverse supports and provide high reaction rates (Qureshi et al. 2005; Lim et al.

2006). On the other hand, of the solid supports used, activated carbon offers the advantages of a large adsorptive capacity and an irregular shape, and shelters bacteria from high fluid shear forces (Moreno-Castilla 2003). Nevertheless, commercially available activated carbons are expensive so it is better to find low-cost solid carriers for use in wastewater treatment production of activated carbons from available sources. Research, then, has mainly focused on producing activated carbons from such sources as waste from the production of cereals, nut shells, olive stones, etc. (Dias et al. 2007). Sewage sludge is also potentially appropriate for the production of carbons because of its wide availability, carbonaceous structure, low cost, and rich content of organic materials (Ocampo-Pérez et al. 2012). For this reason, many studies using sewage sludge as catalytic support material have focused on the production of CMs for adsorbing organic pollutants (Ros et al. 2006; García-Martínez et al. 2015; Athalathil et al. 2014).

In this chapter, a continuous anaerobic up-flow stirred packed-bed reactor filled with biological sludge carbonaceous material (USPBR-BSCM) was applied for the reduction of nitrate. This system was compared with a continuous USPBR filled with AC, as catalytic support material for nitrate removal, in order to study the effectiveness of the new tested carbon material. In addition, the influence of different factors in the biological beds and reactor systems was investigated, in order to demonstrate their promising application for nitrate removal.

4.2. Denitrification in continuous USPBR

In continuous USPBRs the decisive factor is the quantity of catalyst rather than the volume of the reactor; therefore, is more suitable to consider conversion values as a function of space time instead of hydraulic residence time (HRT).Thus, the space time (τ, min) is defined by the following equation:

$$\tau = \frac{m_c}{F_v \cdot \rho} \qquad \text{(eq. 4.1)}$$

where m_c (g) is the amount of catalyst in the reactor, F_v (ml min^{-1}) is the volumetric flow rate of nitrate solution, and ρ (g ml^{-1}) is the density of the solution (Mezohegyi et al. 2007).

In USPBR-BSCM and USPBR-BAC the denitrification was started at a space time (τ) of 2 min (HRT of 6min) in both bioreactors. The results are shown in figure 4.1. For ten days of continuous-flow, effluent nitrate was lower than 25 $mgNO_3^-$/L and nitrate removal was about 60% for both systems. After some fluctuations during the first 20 days of continuous operation, the reactors reached steady state and the processes were controlled for another 55 days. The nitrate and nitrite concentrations in the effluent of USPBR-BSCM fluctuated between 0,50–32,50 $mgNO_3^-$/L and 0,30–0,86 $mgNO_2^-$/L, respectively; the removal rates for nitrate were 35,64 gNO_3^- L^{-1} d^{-1} (0,57 $molNO_3^-$ L^{-1} d^{-1}). In USPBR-BAC the nitrate and nitrite concentrations in the effluent, for the same period of operation, fluctuated between 0,50–29,50 $mgNO_3^-$/L and 0,36–0,86 $mgNO_2^-$/L, respectively; the removal rates for nitrate were

34,92 gNO_3^- L^{-1} d^{-1} (0,56 $molNO_3^-$ L^{-1} d^{-1}). The ammonium concentrations in influent and effluent samples were also monitored for both systems, because of the addition of a huge amount of this compound to the model water (64,40 $mgNH_4^+$/L) as micronutrient for microorganisms. As we can see in figure 4.1a and 4.1b, the consumption of this compound in the bioreactors is very low.

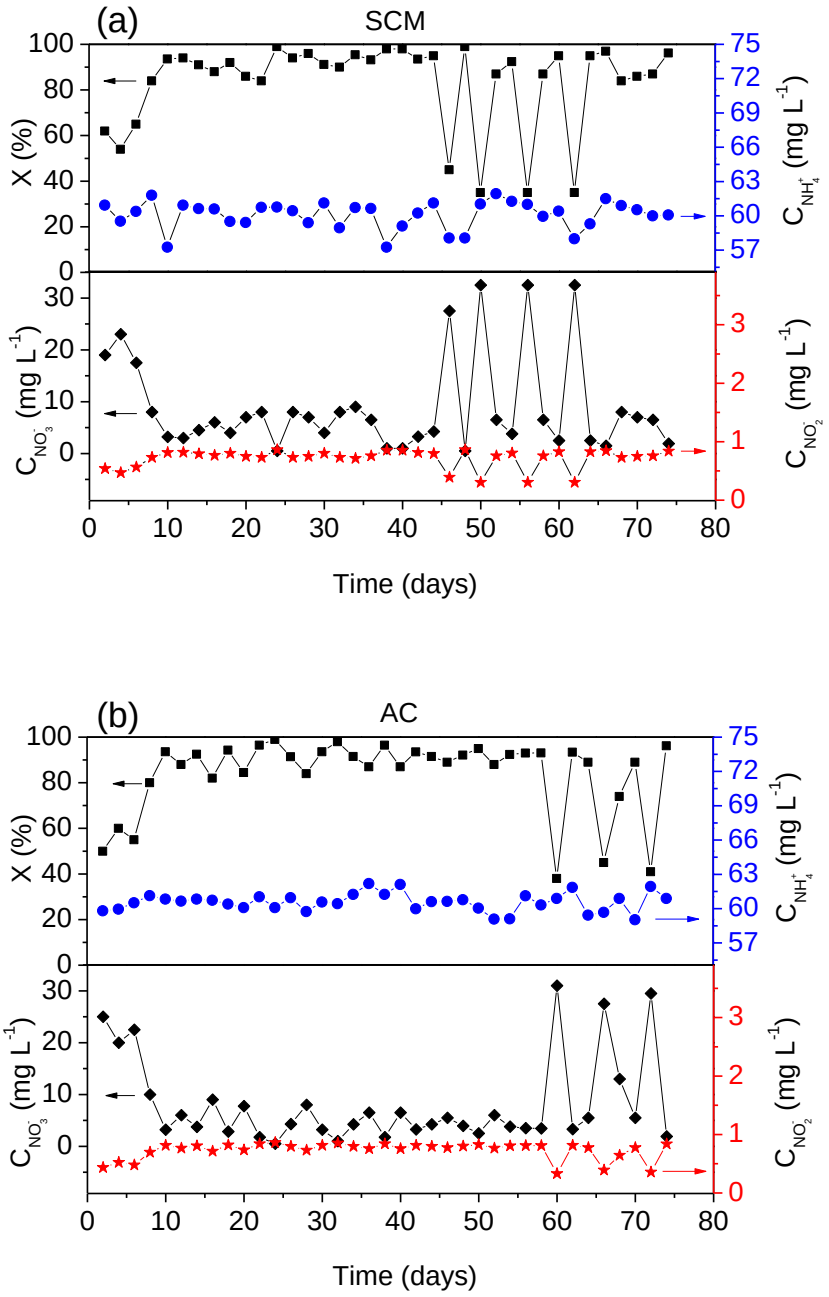

Figure 4.1. Nitrate (◆), nitrite (★), ammonium (●) content of the bioreactor effluent and nitrate removal (■). (a) Continuous USPBR-BSCM system, (b) Continuous USPBR-BAC system.

Nitrate removal in USPBR-BSCM and USPBR-BAC was about 95-99% for this short space time in the systems working in continuous. Table 4.1 compares

these results with those of other denitrification bioreactors reported in the literature. It seems that USPBR-BSCM and USPBR-BAC require the shortest time to accomplish almost complete denitrification. Foglar (Foglar 2013) studied the use of natural zeolite interacted with bacterial cells to obtain bio-zeolite particles for nitrate removal, in a continuous-flow stirred reactor. The removal rate of nitrate was 7,97 gNO_3^- L^{-1} d^{-1} (0,13 $molNO_3^-$ L^{-1} d^{-1}) at a hydraulic residence time (HRT) of 1,32 h. Kesserű (Kesserű et al. 2003) evaluated biological denitrification in a continuous-flow pilot bioreactor containing immobilized *Pseudomonas butanovora* cells. The average removal rates were 3,90 gNO_3^- L^{-1} d^{-1} (0,004 $molNO_3^-$ L^{-1} d^{-1}) and 2,39 gNO_3^- L^{-1} d^{-1} (0,002 $molNO_3^-$ L^{-1} d^{-1}) at ethanol–C:nitrate–N ratios of 3:1 and 1.5:1, respectively. Montalvo (Montalvo et al. 2014) modified an up-flow anaerobic sludge blanket (UASB) reactor using zeolite to improve the nitrate removal process. The reactor achieved a nitrate removal efficiency of 92,4% and a removal rate of 6,2 gNO_3^- L^{-1} d^{-1} (0,10 $molNO_3^-$ L^{-1} d^{-1}) at HRT of 2,5 h. Isaka (Isaka et al. 2012) evaluated the nitrate removal performance of polyethylene glycol (PEG) gel carriers containing entrapped heterotrophic denitrifying bacteria in a cylindrical reactor. A maximum nitrate removal rate of 22,58 gNO_3^- L^{-1} d^{-1} (0,36 $molNO_3^-$ L^{-1} d^{-1}) was observed. Rabah and Dahab (Rabah & Dahab 2004) investigated the nitrate removal characteristics of high performance fluidized-bed biofilm reactors (FBBR) with sand as biofilm carrier. Complete mineralization was observed at a rate of 27,90 gNO_3^- L^{-1} d^{-1} (0,45 $molNO_3^-$ L^{-1} d^{-1}) at an HRT of 3,8 h. Jing (Jing et al. 2009) studied the performance of an anaerobic reactor for simultaneous sulfide and nitrate

removal. The removal rate for nitrate was 4,60 gNO_3^- L^{-1} d^{-1} (0,07 $molNO_3^-$ L^{-1} d^{-1}) at an HRT of 4 h. Tavares (Tavares de Sousa et al. 2009) tested an alternative system, in which anaerobic digestion and denitrification take place in the same UASB. The removal rate was 0,19 gNO_3^- L^{-1} d^{-1} (0,003 $molNO_3^-$ L^{-1} d^{-1}) at an HRT of 6 h. Barber (Barber 2000) carried out the nitrate removal in an anaerobic baffled reactor (ABR). Nitrate removal efficiency was 82% and removal rate was 0,98 gNO_3^- L^{-1} d^{-1} (0,02 $molNO_3^-$ L^{-1} d^{-1}) with a very high HRT of 20 h. Cai (Cai et al. 2015) proposed using methane in biogas as an electron donor to facilitate complete nitrate removal with denitrifying anaerobic methane oxidizing (DAMO) microorganisms, in an anaerobic ammonium oxidation (Anammox) reactor. The nitrate removal rate was 3,03 gNO_3^- L^{-1} d^{-1} (0,05 $molNO_3^-$ L^{-1} d^{-1} at an HRT of 36 h. Waki (Waki et al. 2005) developed a semi-partitioned reactor to study methane-dependent denitrification. The removal rate was 0,27 gNO_3^- L^{-1} d^{-1} (0,004 $molNO_3^-$ L^{-1} d^{-1}) at an HRT of 72 h. Islas-Lima (Islas-Lima et al. 2004) obtained a high nitrate removal rate of 31,34 gNO_3^- L^{-1} d^{-1} (0,51 $molNO_3^-$ L^{-1} d^{-1}) in a continuous stirred tank reactor (CSTR). However, the HRT was 48h.

These results are important because AC is costly and using CMs from exhausted-sludge materials as catalysts is an advantage for biological processes. The high nitrate removal rate and efficiency obtained in this study reveal that the use of SCM for denitrification involves a complex process in which biology, chemistry and physics have a synergetic effect.

Table 4.1. Results of studies reported in the literature by several denitrifying reactors.

Reactor type	Concentration ($mgNO_3^- L^{-1}$)	Rate ($mgNO_3^- L^{-1} d^{-1}$)	Nitrate removal (%)	References
Continuous-flow stirred reactor with zeolite	443	7968	99	(Foglar & Gašparac 2013)
Continuous-flow pilot bioreactor with *Pseudomonas butanovora*	50	3896 2390	100	(Kesserű et al. 2003)
Up-flow anaerobic sludge blanket reactor	2300	6200	92.4	(Montalvo et al. 2014)
Cylindrical reactor with gel carrier cubes	2430-4860	22577	92	(Isaka et al. 2012)
Fluidized bed biofilm reactor	4427	27903	99.8	(Rabah & Dahab 2004)
Anaerobic up-flow reactor with biomass Retention	95.5	4604	N.R	(Jing et al. 2009)
Up-flow anaerobic sludge blanket reactor	53	191	90	(Tavares de Sousa et al. 2009)
Anaerobic baffled reactor	1000	984	82	(Barber 2000)
Anaerobic ammonium oxidation reactor	4427	3028	92	(Cai et al. 2015)
Semi-partitioned reactor mixed cultures	1210	266	N.R	(Waki et al. 2005)
Continuous stirred tank reactor	600	31342	99.3	(Islas-Lima et al. 2004)
USPBR-BSCM	50	35640	95-99	This study

4.3. Effect of slow agitation in USPBR-BSCM and USPBR-BAC

After the packed-bed reactors had been in operation for some time, nitrate conversion started to decline slowly. This behaviour can be observed in figure 4.1a and 4.1b on days 45 and 60 in USPBR-BSCM and USPBR-BAC, respectively. It is caused by the isolation of metabolically active organisms on the sludge carbonaceous material surface and activated carbon with continuous expansion of biofilm over the catalysts (Scholz & Martin 1997). Therefore, BSCM and BAC were slowly agitated in the bioreactors.

The influence of slow agitation was assayed in three up-flow stirred packed-bed reactors, two of which were filled with activated carbon as catalytic support material and the other with sludge carbonaceous material. Figure 4.2 shows nitrate conversion over the time. In the first 20 days, the three bioreactors were continuously fed so that steady state could be reached. Slow agitation was applied in USPBR-BAC1 on day 60, when a decrease in nitrate conversion had been observed for the first time. It can clearly be seen that nitrate conversion increased when slow agitation was applied in the bioreactor. In USPBR-BAC2 slow agitation was first applied on day 55 in an attempt to prevent nitrate conversion from decreasing. In USPBR-BSCM a decrease in nitrate conversion was observed on day 45. Once agitation was stopped, conversion started to decrease in the bioreactors, which showed the positive influence that stirring BSCM and BAC has on nitrate removal. Nevertheless, conversions of nitrate were different before stirring was applied in the three bioreactors, in the same space time (2min). This is because there were different amounts of biomass in

the bioreactors, so it is difficult to control the growth of biomass in biological beds.

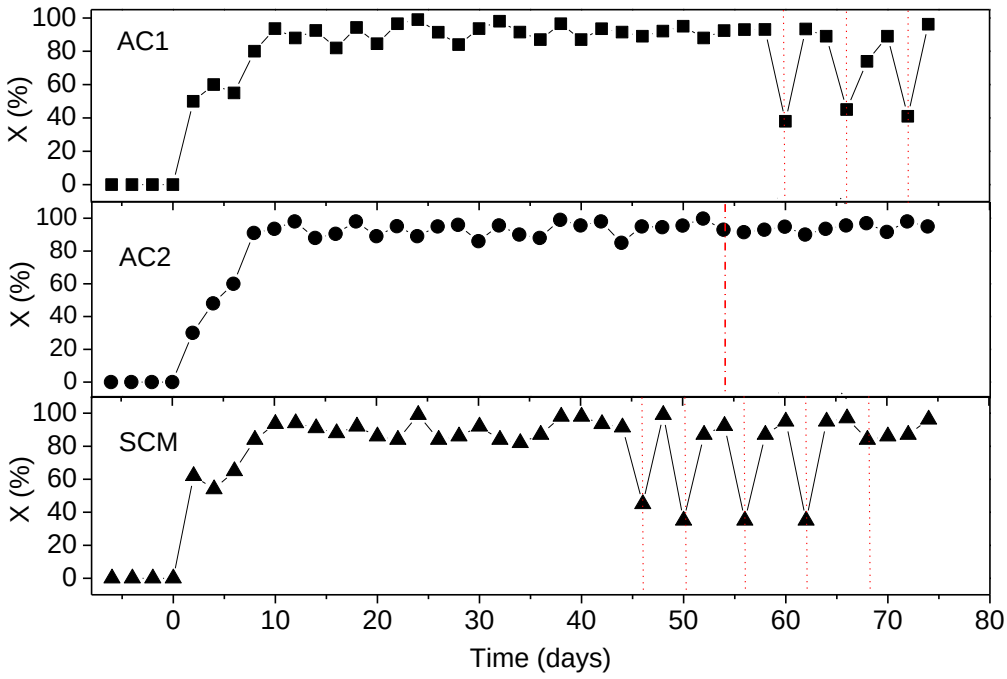

Figure 4.2. Effect of slow agitation in three USPBRs; two filled with activated carbon as catalytic support material (USPBR-BAC1 and USPBR-BAC2) and other with sludge carbonaceous material (USPBR-BSCM): conversion (X); dash line shows the applied agitation in certain days.

For all these reasons, slow agitation of BSCM and BAC together with a continuous flow of nitrate solution within the packed bed can help decrease the unnecessary amount of biomass from the bioreactor. Agitation can also ensure that the amount of biomass in the packed bed is approximately continuous, assuming that there is no significant activated carbon or sludge carbonaceous

material wash-out (the reactor systems were built with two filters, at the top and bottom of the bioreactors, to prevent the carbon materials from washing out). This improves the performance of the SCM and AC.

4.4. Influence of nitrate concentration on biological denitrification

The denitrification rate and nitrate removal as a function of nitrate concentration are shown in figure 4.3. Between 25 and 100 mgNO_3^-/L, the denitrification rate increased and nitrate removal decreased with increasing nitrate concentration. As shown in this figure, similar trends were observed in the two sets of experiments. The highest denitrification rate and nitrate removal obtained in our study in USPBR-BSCM were $3{,}67 \cdot 10^{-3}$ mmol$NO_3^-$$min^{-1}g^{-1}$ and 99%, respectively, while in USPBR-BAC they were $2{,}04 \cdot 10^{-3}$ mmol$NO_3^-$$min^{-1}g^{-1}$ and 97%, respectively. The values indicate that nitrate removal by BSCM is a little higher than in BAC.

For both systems, the shape of the denitrification rate curve is very much like the curvature obtained by Vrtovšek and Roš (Vrtovšek & Roš 2006) in a biofilm reactor with a mixture of PVC plastic and powdered activated carbon as the support material. Similar results were also reported by Lee (Lee et al. 2004) in a columnar packed-bed (PB) reactor with glass raschig rings as the support material.

On the other hand, Rabah (Rabah & Dahab 2004) found that nitrogen removal decreased when the nitrogen loading rate increased. We observed similar behaviour in our systems when the nitrate concentration increased.

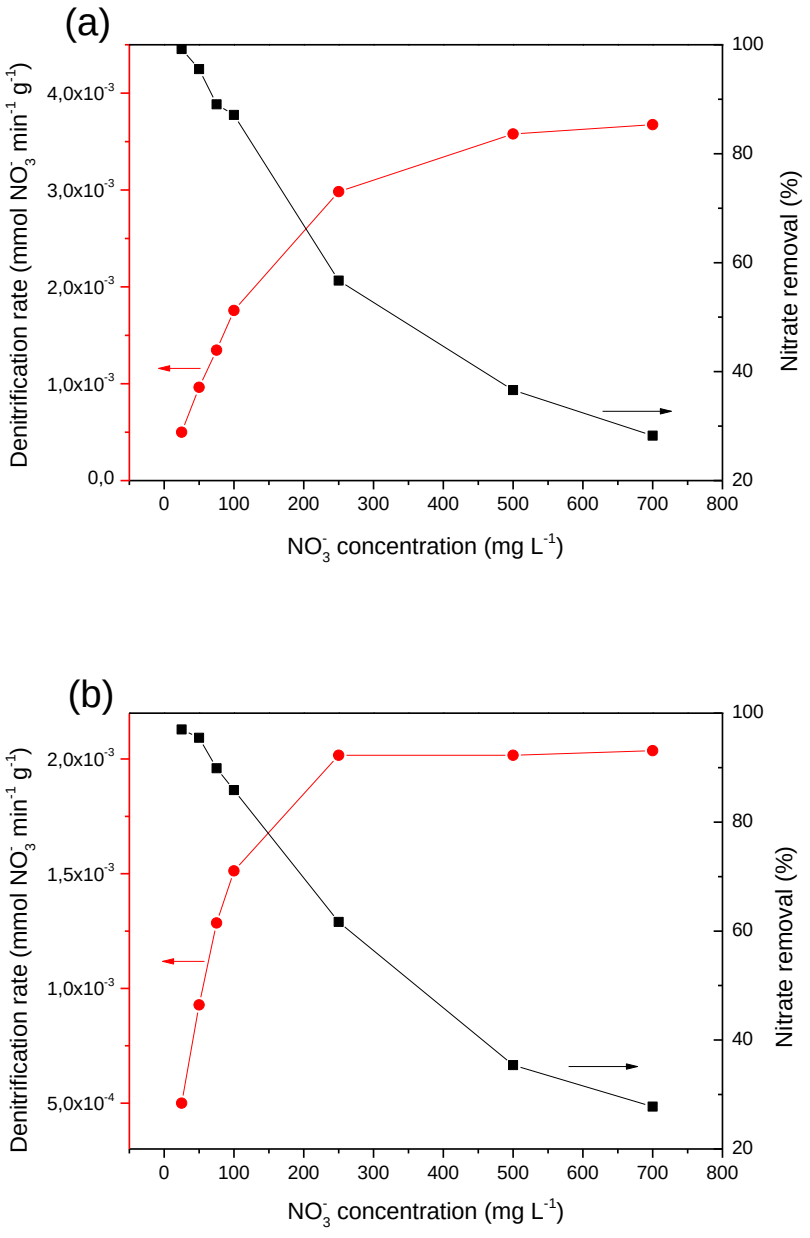

Figure 4.3. Denitrification rate (●) and nitrate removal (■) versus nitrate concentration. (a) USPBR-BAC, (b) USPBR-BSCM.

4.5. Denitrification kinetic analysis

In packed-bed reactors the mole balance is given by equation 4.2:

$$\frac{dF_{NO_3^-}}{dm_c} = -r_{NO_3^-} = \frac{d(C_{NO_3^-} \cdot F_v)}{d(\tau \cdot F_v \cdot \rho)} \quad \text{(eq. 4.2)}$$

where $F_{NO_3^-}$ (mmol min^{-1}) is the molar flow of nitrate solution, m_C (g) is the amount of catalyst in the bioreactor, $C_{NO_3^-}$ ($mmolL^{-1}$) is the nitrate concentration, F_v is the volumetric flow, τ(min) is the space time, ρ(g L^{-1}) is the density of solution and $r_{NO_3^-}$ (mmol min^{-1} g^{-1}) is the nitrate removal rate. If the density of the solution is equivalent to the density of water and the flow rate of nitrate solution is kept constant, the denitrification rate will finally be:

$$\frac{dC_{NO_3^-}}{d\tau} = r_{NO_3^-} \quad \text{(eq. 4.3)}$$

Furthermore, the Michaelis–Menten model is expected to adequately describe the denitrification process in the USPBRs. The kinetic rate according to Michaelis-Menten model is as follows (Li et al. 2001):

$$r_{NO_3^-} = \frac{dC_{NO_3^-}}{d\tau} = -\frac{k_1 \cdot C_{NO_3^-}}{k_2 + C_{NO_3^-}} \quad \text{(eq. 4.4)}$$

where k_1 ($mmolL^{-1}$ min^{-1}) is the maximum removal rate and k_2 (mmol L^{-1}) is the half maximum rate.

Equation 4.4 was solved using MATLAB® R2012a. The algorithm uses the fifth-order Runge-Kutta subroutine to solve the differential equation coupled to a nonlinear least-squares fitting algorithm (lsqnonlin; algorithm: Trust-Region-Reflective), to estimate the values of the parameters in the equation.

Nitrate reduction in the USPBR-BSCM and USPBR-BAC systems can be described by the Michaelis–Menten model (equation 4.4), since it involves heterogeneous catalysis and biological processes. The model fits the experimental results well for concentrations below 250 mg/L (see figure 4.4). Similar results were obtained by Foglar (Foglar et al. 2005) for initial nitrate concentrations above 400 $mgNO_3^-$-N/L, in which the nitrate removal predicted by the Michaelis-Menten model is a curve rather than a straight line. Foglar states that denitrification is a complex process and, therefore, that assuming certain parameters and simplifying the model can have some drawbacks for the original model. Table 4.2 shows the kinetic parameters encountered for this model. As can be seen, the maximum removal rate for USPBR-BSCM (k1= 3,37 $mmolNO_3^-L^{-1}min^{-1}$) is similar to the maximum rate for USPBR-BAC (k1= 2,50 $mmolNO_3^-L^{-1}min^{-1}$), which confirms that SCM is a powerful adsorbent that can efficiently remove nitrates from wastewaters. Using the Michaelis-Menten equation for calculating kinetic constants with data from batch experiments, Foglar (Foglar et al. 2007) estimated the denitrification kinetics in a bioreactor filled with zeolite as carrier material. The kinetic parameters obtained were k_1= 0,0844 $mgNO_3^-$-N L^{-1} h^{-1} (1 x 10^{-4} $mmolNO_3^-$ L^{-1} min^{-1}) and k_2= 5,18 x 10^{-5} $mgNO_3^-$-N L^{-1} (3,7 x 10^{-6} $mmolNO_3^-$ L^{-1}). Cao (Cao et al. 2002) studied denitrification kinetics in a bubble-column bioreactor filled with a gel matrix of polyvinyl alcohol (PVA) to co-immobilize denitrifying bacteria. They

certified that the single-stage bio-denitrification process obeyed Michaelis-Menten kinetics. The kinetic parameters for nitrate removal were found to be 34,5 $mgNO_3^-$-N L^{-1} h^{-1} (0,04 $mmolNO_3^-$ L^{-1} min^{-1}) and k_2= 303 $mgNO_3^-$-N L^{-1} (21,6 $mmolNO_3^-$ L^{-1}). Dinçer (Dinçer & Kargı 2000) studied the kinetics of nitrification and denitrification of synthetic wastewater using two reactors in series: an activated sludge unit for nitrification followed by a down-flow bio-filter for denitrification. The experimental data indicated that denitrification kinetics followed the Michaelis-Menten model and the kinetic parameters for denitrification were found to be k_1= 280 $mgNO_3^-$-N L^{-1} h^{-1} (0,01 $mmolNO_3^-$ L^{-1} min^{-1}) and k_2= 0,27 $mgNO_3^-$-N L^{-1} (0,02 $mmolNO_3^-$ L^{-1}). Therefore, to the best of our knowledge, denitrification in USPBRs, mainly USPBR-BSCM, is the fastest anaerobic nitrate removal process ever reported, which confirms that SCM can be an efficient alternative for producing low-cost AC and an effective waste management practice.

Table 4. 2. Kinetic parameters of Michaelis-Menten model in USPBR-BSCM and USPBR-BAC.

Reactor	**k_1 (mmol $NO_3^-L^{-1}min^{-1}$)**	**k_2 (mmol $NO_3^-L^{-1}$)**	**σ^a**
USPBR-BSCM	3,37	0,58	0,22
USPBR-BAC	2,50	0,27	0,34

[a]Standard deviation associated with the model fitting: $\sigma = \frac{\sqrt{\sum(X - X^{MOD})^2}}{n-1}$, where n is the number of experimental points.

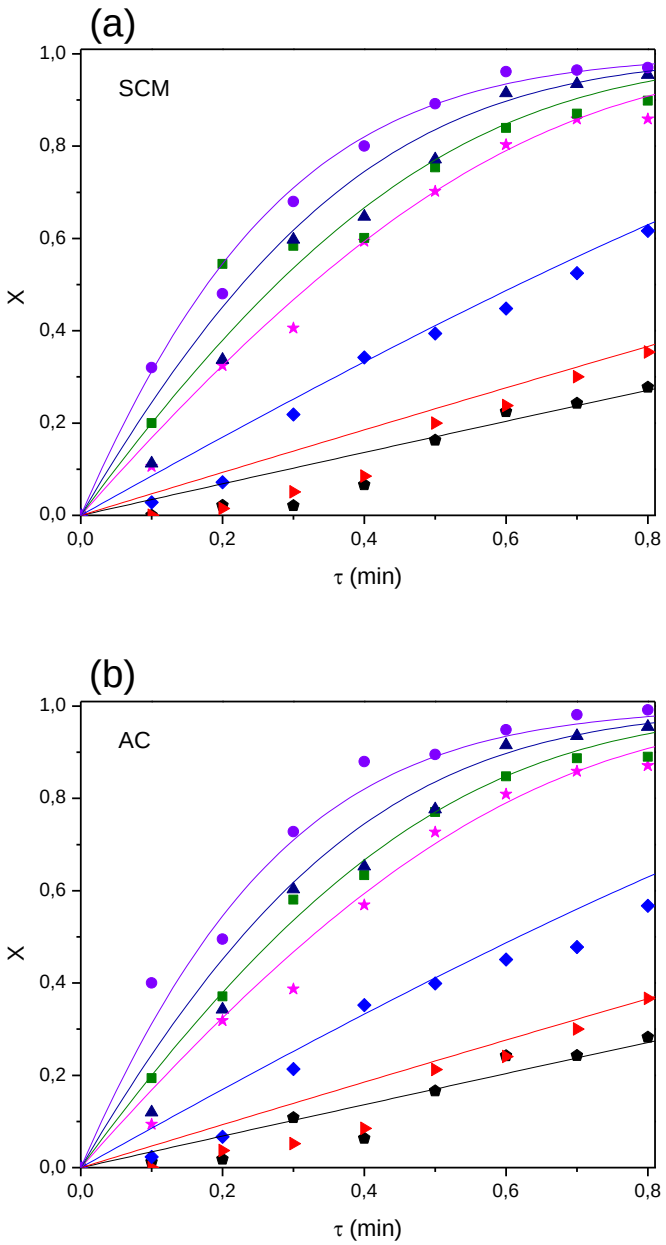

Figure 4.4. Kinetic modelling of nitrate removal in (a) USPBR-BSCM, (b) USPBR-BAC. Nitrate concentrations: 25 mg·L^{-1} (◆), 50 mg·L^{-1} (▶), 75 mg·L^{-1} (●), 100 mg·L^{-1} (⬟), 250 mg·L^{-1} (▲), 500 mg·L^{-1} (■), 700 mg·L^{-1} (★); lines represent the fit to the Michaelis-Menten model.

4.6. Conclusions

The anaerobic removal of nitrates in a continuous USPBR containing BSCM demonstrated the effectiveness of the process and their promising application for the biodegradation of this pollutant. The carbonaceous material (CM), obtained from exhausted sludge, was used as support to grow the biofilm and solid electron mediator for nitrate reduction in the bioreactor. In a continuous USPBR-BSCM system, nitrate removal efficiency was 99% at very short space times (τ) 2 min/hydraulic residence times (HRT) 6 min. By comparing these results with the hydraulic residence times applied in other classical denitrification technologies, it seems that USPBR-BSCM requires the shortest times to achieve almost complete nitrate removal. In addition, a high denitrification rate of $3{,}67 \cdot 10^{-3}$ mmolNO^{3-} min^{-1} g^{-1} was observed in the USPBR-BSCM, whilst in the USPBR-biological activated carbon (BAC) it was $2{,}04 \cdot 10^{-3}$ mmolNO^{3-} min^{-1} g^{-1}. The kinetic analysis of the systems showed a good fit with the Michaelis–Menten model. Results indicate that SCM is a potentially low cost catalyst and effectively competes with commercial ones. Thus, USPBR-BSCM is an inexpensive and advantageous process for nitrate removal and also a very effective and promising system for denitrification.

Chapter 5

Removal of CB, 2,4-D and IBP in anaerobic packed-bed reactors

5.1. Introduction

The attached growth technique is a hopeful option for wastewater remediation, and comprises the attached growth over inert carriers. It presents several advantages, such as high biomass concentrations, effectiveness for the removal of inorganic compounds, can be employ for high organic loading rates at fairly shorter HRT, fewer subject to unsteady or irregular loadings, small reactor size and low operational costs (Guo et al. 2012).

The biological packed-bed reactor is regarded to be a great prototype technique for the removal of recalcitrant pollutants, because of in it the biological degradation of different wastewaters can be modelling and have been convincingly utilized for the removal of numerous organic chemicals, and their possible breakdown products (González et al. 2006).

On the whole, in spite of the attached growth systems, especially the packed-bed reactor, have not been widely and expressly used for the removal of CB , 2,4-D and IBP, some recent research results have proven that this process is a promising technique to lower the release of several emergent pollutants into the environment. Additionally, the use of packing carriers helps to raise the microbial community in the reactor, and keep it retain in the system; facilitating the growth of slow-growing microorganisms for the degradation of recalcitrant compounds (Serrano et al. 2011). Thus, based on the broad finding in the environment, wide use and scarce data available in the literature on the removal of CB, 2,4-D and IBP, the principal objective of this chapter was to study the

anaerobic degradation of CB, 2,4-D and IBP by means of an up-flow stirred packed-bed reactor. The adsorption capacity of the granular activated carbon (GAC), used as carrier material, for the target pollutants have been also investigated.

5.2. Adsorption isotherms

Figure 5.1 shows the experimental isotherms for the adsorption of the CB, 2,4-D and IBP, from single component solutions, over the activated carbon at 35ºC. The adsorption of organic compounds on granular activated carbon (GAC) in water is better described by Langmuir adsorption isotherm model than other models (Mestre et al. 2007; Tsai et al. 2006); therefore, Langmuir model was used to test the experimental data and can be given as the following equation:

$$Q_e = \frac{Q_L \cdot K_L \cdot C_e}{1 + K_L \cdot C_e} \qquad \text{(eq. 5.1)}$$

Where Q_e (mg g_{AC}^{-1}) and C_e (mg L^{-1}) are the values of the concentrations in the solid and liquid phases at the equilibrium, respectively. Q_L (mg g_{AC}^{-1}) is the maximum adsorption capacity, in accordance with the Langmuir model and K_L (L mg^{-1}) is the Langmuir constant. From the figure, it is observed that the experimental data showed excellent fitting with the model, so the maximum adsorption capacity of each studied micropollutant (CB, 2,4-D and IBP) was found by using the linearized form of equation 5.1 and is compiled in table 5.1. Moreover, the blank control of CB was less than 3 % of sample loss as seen during the experiments, corroborating the high quality of adsorption analysis.

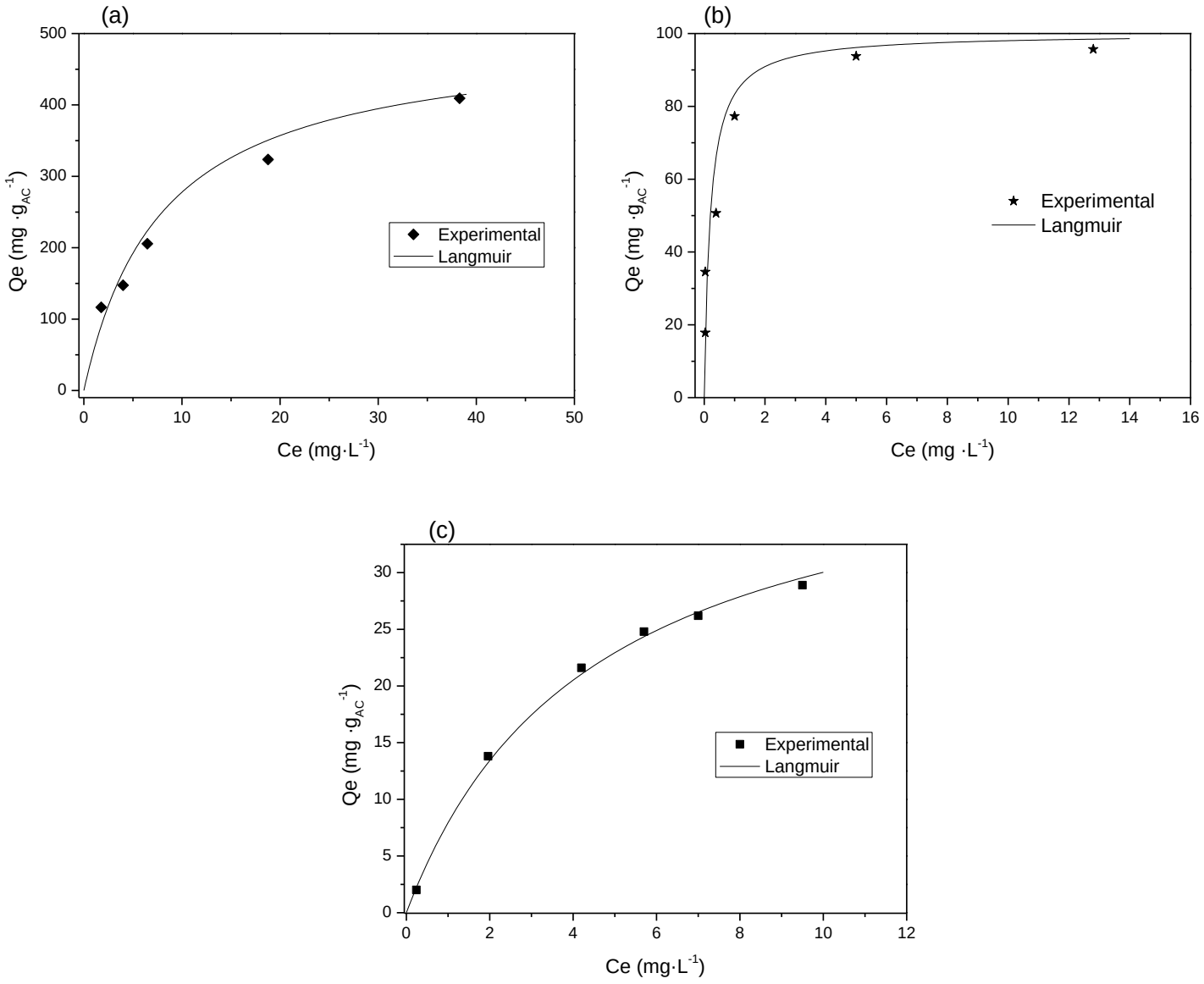

Figure 5.1. Equilibrium adsorption isotherms of CB (a), 2,4-D (b) and IBP (c) from single component solutions over activated carbon (35ºC). Symbols represent experimental data and lines are the fitting to the Langmuir equation.

Table 5.1. Fitting parameters of the equilibrium adsorption isotherms to the Langmuir model.

Compound	**Q_L (mgg^{-1})**	**K_L ($L\ mg^{-1}$)**	**R^2**
CB	417	0,15	0,99
2,4D	100	5,00	0,99
IBP	29	0,22	0,99

The adsorption isotherms of CB, 2,4-D and IBP correspond to type L of Giles classification, displaying an initial ascent and a concave curvature at low equilibrium concentrations followed by a well-defined plateau (which is the saturation limit). This is distinctive of a high affinity of the adsorbates for the solid phase, without an strong competition of the solvent and almost insignificant interactions between the adsorbed molecules; therefore, the adsorption proceeds by the formation of a monolayer in the interval of concentrations used (Giles et al. 1960; Mansouri et al. 2015).

Additionally, the maximum adsorption capacities obtained for CB, 2,4-D and IBP are similar to those reported in the literature for carbon adsorbents. Oliveira et. al (Oliveira et al. 2002) studied the adsorption of volatile organic compounds in water over activated carbon/iron oxide magnetic composites; for CB the maximum adsorption capacity was about 305 mg/g at 25 °C and experiments with pure activated carbon showed an adsorption capacity of 480mg/g. Salman et. al (Salman & Al-Saad 2012) evaluated the adsorption potential of the date seeds based activated carbon in removing 2,4-D from aqueous solution; the equilibrium data were best represented by Langmuir isotherm model, showing

maximum monolayer adsorption capacity of 175,4 mg/g at 30 °C. Baccar and coworkers (Baccar et al. 2012) investigated the adsorption of ibuprofen and other drugs onto low-cost activated carbon, the maximum adsorption capacity determined from the Langmuir model was 12,6 mg g^{-1} for IBP.

5.3. Biodegradation of CB, 2,4-D and IBP in continuous experiments

Continuous experiments were accomplished to study the biodegradation of CB, 2,4-D and IBP in anaerobic USPBRs; as it was mentioned in Chapter 4 (session 4.2) in the case of continuous packed-bed reactors working with catalysts, is more adequate to examine conversion values as a function of space time. Before beginning the experiments in continuous USPBRs, the flow rate of the feeding solution was increased 3 times more and pumped within the reactors to prevent the adsorption effects. Initially, a decreased in the conversion values of CB and 2,4-D was observed but after almost 48 h of continuous flow, the concentration of the contaminants in the outlet solution was equal to the inlet solution; confirming the saturation of the carbon bed.

Figure 5.2 shows the starting and stabilization period for USPBRs degrading CB, 2,4-D and IBP at initial space time (τ) of 2min (0,033h). As we can see in the figure, after some fluctuations during the first 50, 60 and 85 days of continuous operation for CB, IBP and 2,4-D respectively, the bioreactors reached the steady state and the processes were monitored over another 20 days, approximately. As shown, the conversion was very low, around 1, 2 and 5 % for IBP, 2,4-D and CB, respectively. These values represents a removal

rates of 0,14 g L^{-1} d^{-1} (0,00063 mol L^{-1} d^{-1}), 0,94 g L^{-1} d^{-1} (0,0083 mol L^{-1} d^{-1}) and 0,96 g L^{-1} d^{-1} (0,0043 mol L^{-1} d^{-1}) for IBP, CB and 2,4-D, respectively. The results indicate that the space time of 0,033h is not enough for the biodegradation of the studied micropollutants. Another important factor affecting the conversion values in USPBR treating 2,4-D was the concentration (100 mg L^{-1}) fed to the bioreactor in the first stage. Some authors selected a low concentration of pollutants to acclimatize and start up the system, thus, the concentration of 2,4-D (25 mg L^{-1}) has been reduced in the feeding solution (Elefsiniotis et al. 2005; Mangat & Elefsiniotis 1999). Once the reactor system has been stabilized, the experiments were continued over. On the other hand, in USPBR used in previous studies by García-Martínez et. al (García-Martínez et al., 2015) a decline of conversion values was noted over the time; which can be a result of the isolation of the microorganisms above the catalysts surface and due to the continuous expansion of biofilm around the solid support (Scholz & Martin 1997). Thereby, taking into account the large period necessary for the USPBRs to reach the steady state and to avoid this problem, a slow agitation on the biological carbon bed was applied. Based on the above observations, figure 5.3 shows the performance of USPBRs at different space times, before and after agitated the bioreactors. As shown, high conversion values were achieved when a slow agitation in the BAC was applied in the bioreactors at several space times. The results confirm that the increase in biomass density can constrain the biodegradation close to the catalyst surface; thus, a slow agitation of the biofilm with a constant flow of micropollutants solution over the carbon bed helps to remove the excess of biomass in the bioreactor. Besides,

the slow agitation can help maintaining a practically constant quantity of biomass in the packed-bed, assuming no meaningful loss of activated carbon.

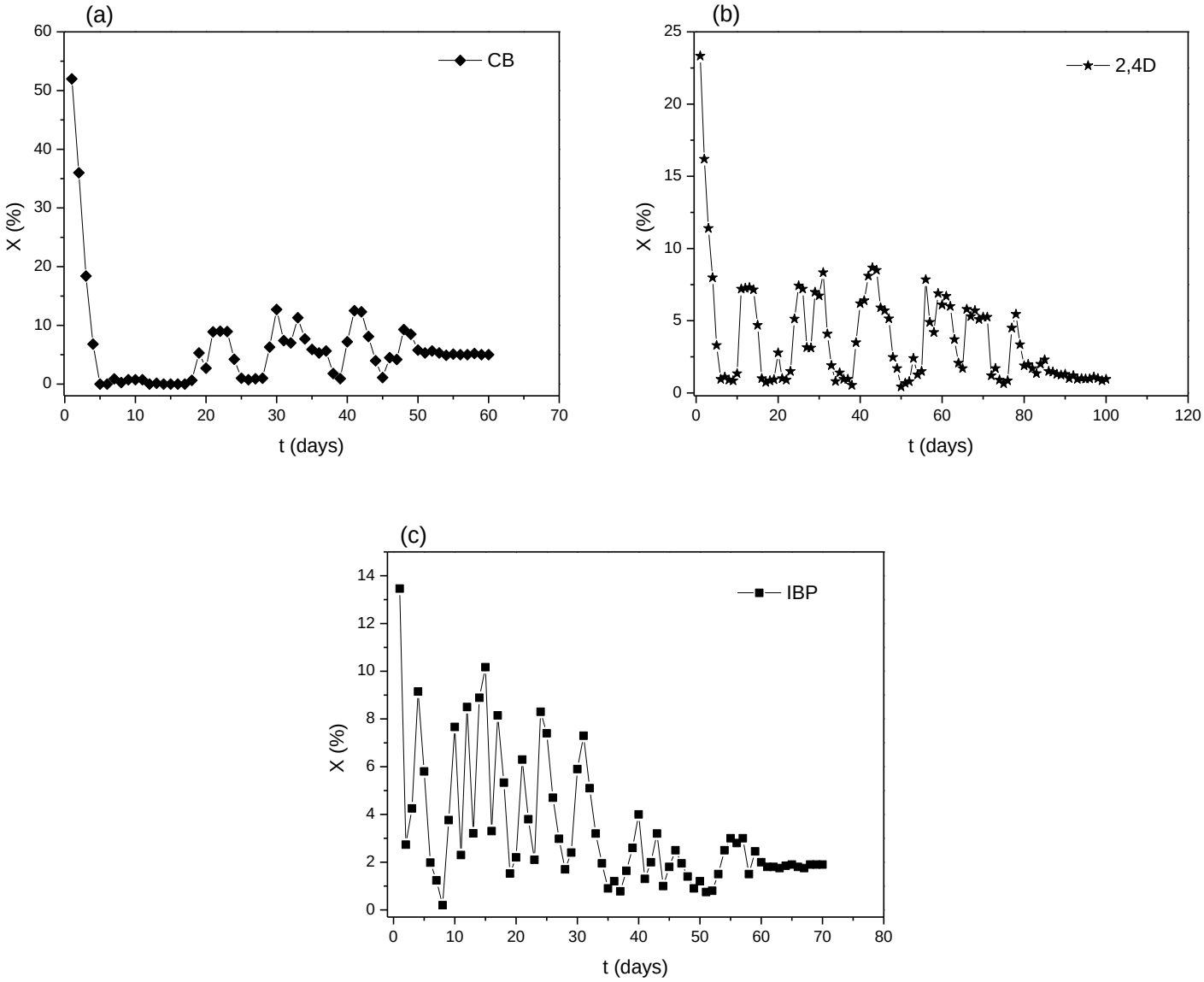

Figure 5.2. Acclimation and stabilization period of USPBRs.

(a) CB (Concentration= 25 mg L^{-1}, T= 35 ºC, τ = 0,033 h),

(b) 2,4D (Concentration= 100 mg L^{-1}, T= 35 ºC, τ = 0,033 h),

(c) IBP (Concentration= 10 mg L^{-1}, T= 35 ºC, τ = 0,033 h)

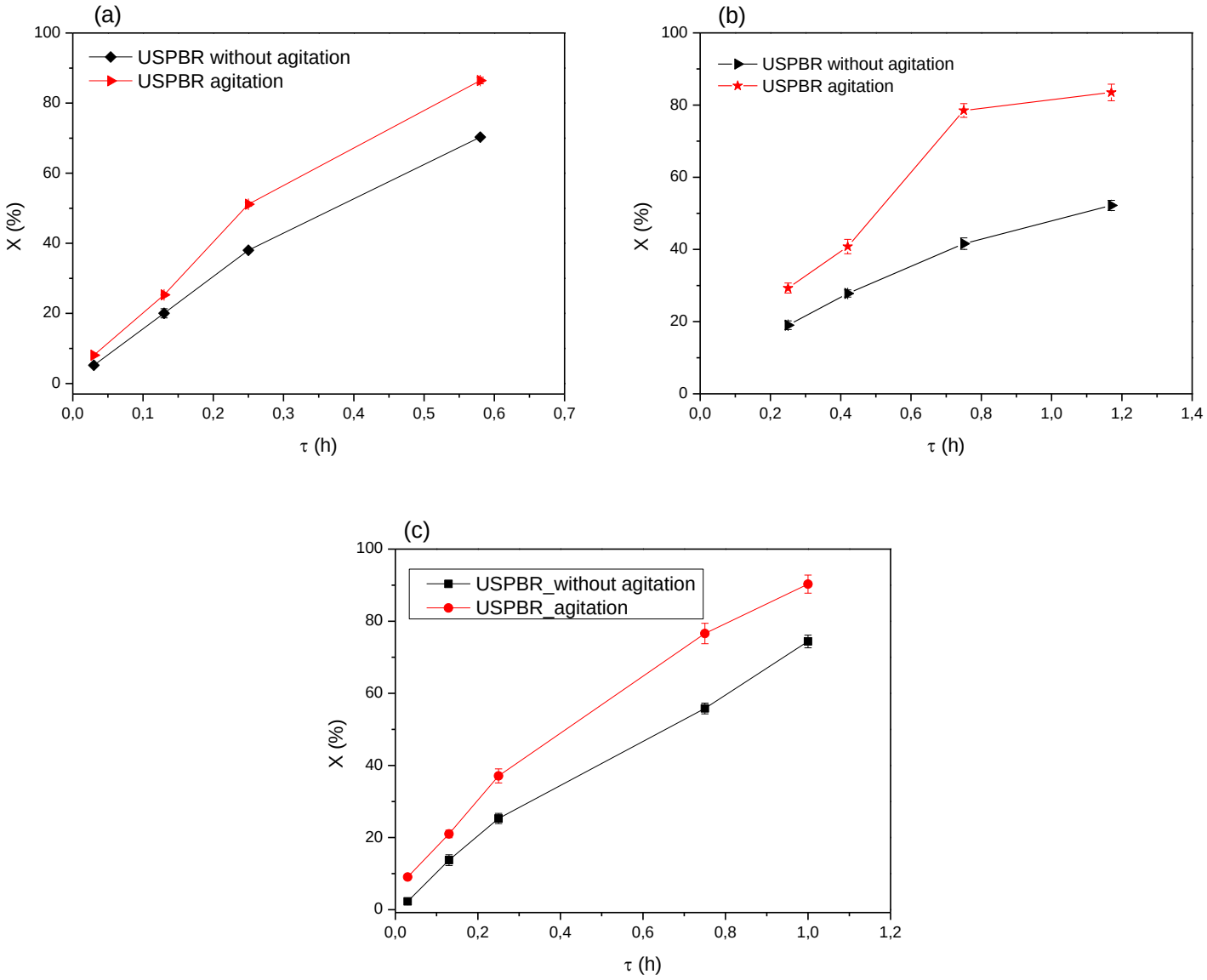

Figure 5.3. Performance of USPBRs at different space times, before and after agitation. (a) CB, (b) 2,4D (Concentration= 25 mg L^{-1}, T= 35 ºC) and (c) IBP (Concentration= 10 mg L^{-1}, T= 35 ºC).

Figure 5.3(a) shows the results for CB, almost 90 % of conversion value was achieved at a space time of 0,58 h (35 min), with a removal rate of 0,89 g L^{-1} d^{-1} (0,0079 mol L^{-1} d^{-1}). By comparing this result with other degradation processes for CB previously reported, it seems that anaerobic USPBR filled with BAC is faster than any other. Emanuelsson and coworkers (Emanuelsson et al. 2005) investigated the microbial community dynamics in a bioreactor inoculated with a

pure bacterial strain (*Burkholderia sp.*) capable of degrading CB; the results revealed that 300 mg L^{-1} of CB was completely degraded with a removal rate of 347 mg L^{-1} d^{-1}. Jechorek et. al (Jechorek et al. 2003) explored the degradation of CB contaminated groundwater in a soil column packed with 13,4 kg aquifer sediments, and colonized with a natural mixed culture of methanotrophic bacteria; the column was highly effective in the removal of CB, reducing the influent concentration of 25–30 mg L^{-1} to 0,04 mg L^{-1}, however, the removal rate was only of 16,5 mg L^{-1} d^{-1}. Wang and coworkers (Wang et al. 2008) studied the CB degradation by electro-heterogeneous catalysis in aqueous solution; the degradation efficiency was almost 100% with an initial concentration of 50 mg/L and the removal rate was up to 800 mg L^{-1} d^{-1}. Moreira et. al (Moreira et al. 2012) studied the co-metabolic degradation of chlorobenzene by the fluorobenzene degrading wild strain *Labrysportucalensis*; the biodegradation of 0,5 mM of CB was achieved at a rate of 7,95 µmol L^{-1} d^{-1} (89 mg L^{-1} d^{-1}).

A different trend is observed for 2,4-D removal. A conversion value around 85 %, was achieved at space time of 1,17 h (70 min) and the removal rate correspond to 0,46 g L^{-1} d^{-1} (0,0021 mol L^{-1} d^{-1}). This tendency reveals that the degradation of CB is faster than the degradation of 2,4-D in USPBRs. However, a comparison with the literature review and the obtained results for 2,4-D degradation demonstrate that the anaerobic USPBR filled with BAC is faster than any other for treating this micropollutant. González et. al (González et al. 2012) studied the degradation of the herbicide 2,4-D by an indigenous *Delftia sp. strain* in batch and continuous systems. Complete degradation of 100 mg L^{-1} of 2,4-D was achieved in 24 h under batch conditions, with a rate of

4,16 mg L^{-1} d^{-1}. In the continuous down-flow fixed-bed reactor using polyurethane foam cubes as a support for immobilizing bacterial cells, the removal rate was 21,7 mg L^{-1} d^{-1}. Quan et. al (Quan et al. 2011) conducted their experiments in a microcosm biofilm reactor operated in fed-batch mode. The biofilm carriers was able to complete degrade an initial concentration of 65 mg L^{-1} of 2,4-D, as the only carbon source, and the removal rate was 56,16 mg L^{-1} d^{-1}. Vroumsia et. al (Vroumsia et al. 2005) studied the fungal bioconversion of 2,4-D and 2,4-dichlorophenol (2,4-DCP). After 5 days of cultivation, the best results were obtained with *Aspergillus penicilloides* for 2,4-D (100 mg L^{-1}), with an efficiency of 50 % and a removal rate of 10 mg L^{-1} d^{-1}. Elefsiniotis and co-worker (Elefsiniotis & Wareham 2013) explored the biodegradability 2,4-D in a laboratory-scale sequencing batch reactor (SBR) that operated under anaerobic conditions. The results revealed that 100 mg L^{-1} of 2,4-D was completely degraded following an acclimation period of 70 d, with a removal rate of 49,5 mg L^{-1} d^{-1}.

For the case of IBP, a maximum conversion value of 90 % was achieved at space time of 1 h (60 min), with a removal rate of 0,22 g L^{-1} d^{-1} (0,00095 mol L^{-1} d^{-1}). The results indicate that this value of space time has an unmeasurable effect on the degradation of IBP and a slow agitation of the biological bed can help eliminating isolated layers of microorganisms, enhancing the performance of the reactor. Choina et. al (Choina et al. 2013) investigated the photo catalytic decomposition of IBP down to low mg/L concentrations over titania catalyst; a percentage removal of 62 % was observed with a removal rate of 0,15 g L^{-1} d^{-1}. Ferrando-Climent and coworkers

(Ferrando-Climent et al. 2012) studied the removal of IBP with different sludge concentrations in batch experiments. The total IBP removal was achieved for experiments performed with high sludge concentration and the removal rate varied between 0,0050-0,048mg L^{-1} d^{-1}. Zwiener and Frimmel (Zwiener & Frimmel 2003) investigated the biodegradation of IBP and two active compounds of pharmaceuticals in short-term tests with a pilot sewage plant (PSP) and biofilm reactors (BFR, oxic and anoxic) as model systems for municipal sewage treatment. The concentration of IBP was decreased to approximately 35% in the oxic BFR and to approximately 40% in the PSP, corresponding to a removal rate of 0,0018 mg L^{-1} d^{-1}. Smooket. al (Smook et al. 2008) made a preliminary study of a biological nutrient removal pilot system (BNR). For initial influent concentrations of 6000 and 12000 ng L^{-1} a removal rate of 0,029 and 0,059 mg L^{-1} d^{-1} were obtained, respectively. Girardi and coworkers (Girardi et al. 2013) compared the biodegradability of IBP and 2,4-D in soil and aqueous systems; 45 % of IBP removal was obtained in the soil system whilst 68 % of removal was obtained in the aqueous system, corresponding to a removal rate of 0,07 and 0,49 mg L^{-1} d^{-1}, respectively.

The high removal rates obtained in this study is due to the combination of different characteristics of the reactor system. Although, packed-bed reactors using BAC have not been commonly employed for anaerobic degradation of the studied micropollutants, the use of microorganisms supported on activated carbon must enhance the fusion of the good capacities of both to retain and/or degrade organic compounds, producing a synergistic effect between biofilm and support (Quintelas et al. 2010). AC presents dissimilar properties that make it

able to enhance the degradation capacity of the system. Firstly, AC is an excellent attachment surface for microorganisms; the porous structure of the carbon particles, provides a protective environment on which microorganisms can settle and colonize easily (Rivera-Utrilla et al. 2003). Secondly, the AC contains surface quinonic structures and their electron transfer properties provide a redox mediating capacity (Van der Zee & Villaverde 2005). This mechanism has been reported previously (Van der Zee et al., 2003), and confirms the role of the AC as redox mediator for the reduction of recalcitrant azo dyes. Whereby, the establishment of the microorganisms on biologically activated carbon (BAC) beds during water treatment can have beneficial effects, due to the efficiency of the combined biodegradation–adsorption process is higher than expected for the processes alone.

Figure 5.4 shows the performance of the USPBRs for long operation periods. The system showed great stability for CB, 2,4-D and IBP during the studied time, up to 40 days. For CB a conversion value around 87 % was achieved at a space time of 0,58 h, whilst for 2,4-D and IBP conversion values around 88 and 90 % were reached at space times of 1,17 and 1 h, respectively.

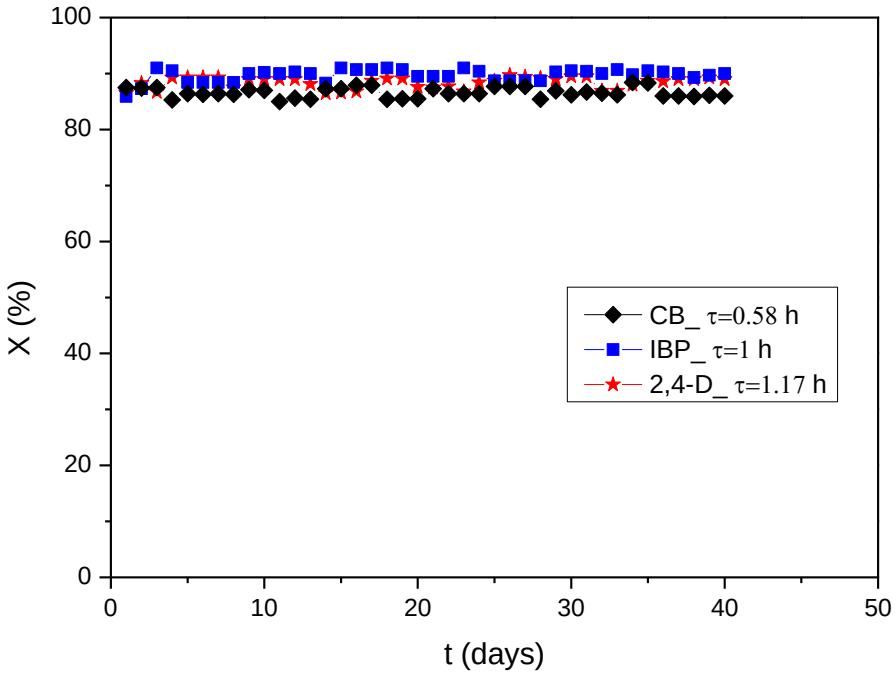

Figure 5.4. Stability of the USPBRs during long operation periods

(Concentration= 25 mg L^{-1} for CB and 2,4-D; 10 mg L^{-1} for IBP, T= 35 ºC).

5.4. Modelling CB, 2,4-D and IBP degradation in USPBRs

The mole balance and the kinetic model used in this study are very similar to the described in Chapter 4 (session 4.5). Just little variations were made in the designation of the variables and are shown below.

$$\frac{dF_P}{dm_c} = -r_P = \frac{d(C_P \cdot F_v)}{d(\tau \cdot F_v \cdot \rho)} \qquad \text{(eq. 5.2)}$$

where F_P (mmol min^{-1}) is the molar flow rate of micropollutant, m_C (g) is the amount of catalyst in the bioreactor, r_P (mmol min^{-1} g^{-1}) is the reaction rate, C_P

(mmol L^{-1}) is the concentration of micropollutant, F_v is the volumetric flow, τ (min) is the space time and ρ (g L^{-1}) is the density of solution.

$$\frac{dC_P}{d\tau} = r_P \qquad \text{(eq. 5.3)}$$

The kinetic rate according to Michaelis-Menten model is as follows:

$$r_P = -\frac{k_1 \cdot C_P}{k_2 + C_P} \qquad \text{(eq. 5.4)}$$

where k_1 (mmol g_{cat}^{-1} min^{-1}) is the maximum rate of micropollutants degradation and k_2 (mmol L^{-1}) is the half velocity constant. Table 5.2 shows the kinetic parameters of the Michaelis-Menten equation and equation 5.4 was solved using MATLAB® R2012a, employing the same algorithm utilized in session 4.5.

Table 5.2. Kinetic parameters for CB, 2,4-D and IBP anaerobic biodegradation in USPBRs with Michaelis-menten model.

Compound	k_1 (mmol g_{cat}^{-1} min^{-1})	k_2 (mmol L^{-1})	σ^a
CB	1,28	0,32	0,003
2,4D	0,098	0,00014	0,05
IBP	0,32	0,15	0,007

[a]Standard deviation associated with the model fitting: $\sigma = \sqrt{\frac{\sum(X - X^{MOD})^2}{n-1}}$, where n is the number of experimental points.

The fitting results displayed in figure 5.5 provide evidence of the goodness of the model which adequately describe the experimental data for CB degradation in USPBR (Figure 5.5(a)), not obtaining a good fitting of the experimental data for the case of 2,4-D and IBP. As can be seen in table 5.2, the maximum rate for CB (k_1= 1,28mmol g_{cat}^{-1} min^{-1}) is higher than the maximum rate for 2,4-D (k_1= 0,098 mmol g_{cat}^{-1} min^{-1}) and IBP (k_1 = 0,32 mmol g_{cat}^{-1} min^{-1}), as supposed according to the commented results. Mathur and coworkers (Mathur et al. 2006) studied the kinetics of the removal of mono-chlorobenzene vapour from waste gases using a trickle bed air biofilter; the Michaelis-Menten model was used as the macro-kinetic determination method. The kinetic parameters obtained were k_1= 0,121 g m^{-3} s^{-1} (0,064 mmol L^{-1} min^{-1}) and k_2= 7,45 g m^{-3} (0,066 mmol L^{-1}). Zhou et. al compared the performance and microbial communities of two differently inoculated bio-trickling filters (BTFs) for treating CB; the kinetic parameters were calculated in order to understand kinetic behaviour of BTFs and the Michaelis–Menten equation was employed for this purpose. The maximum degradation rate (k_1) was 83,61 g m^{-3} s^{-1} (0,74 mmol L^{-1} min^{-1}) for the BTF inoculated with biomass suspension of *Ralstoniapickettii* L2 + the enriched activated sludge, whilst for the BTF inoculated only with the enriched activated sludge k_1= 36,64 g m^{-3} s^{-1} (0,33 mmol L^{-1} min^{-1}); indicating that the introduction of *Ralstoniapickettii* L2 had a positive effect on CB degradation rates of BTF (Zhou et al. 2016). Taking into account the results mentioned above and to the best of our knowledge, the degradation of CB in anaerobic USPBR is the faster biodegradation process ever reported.

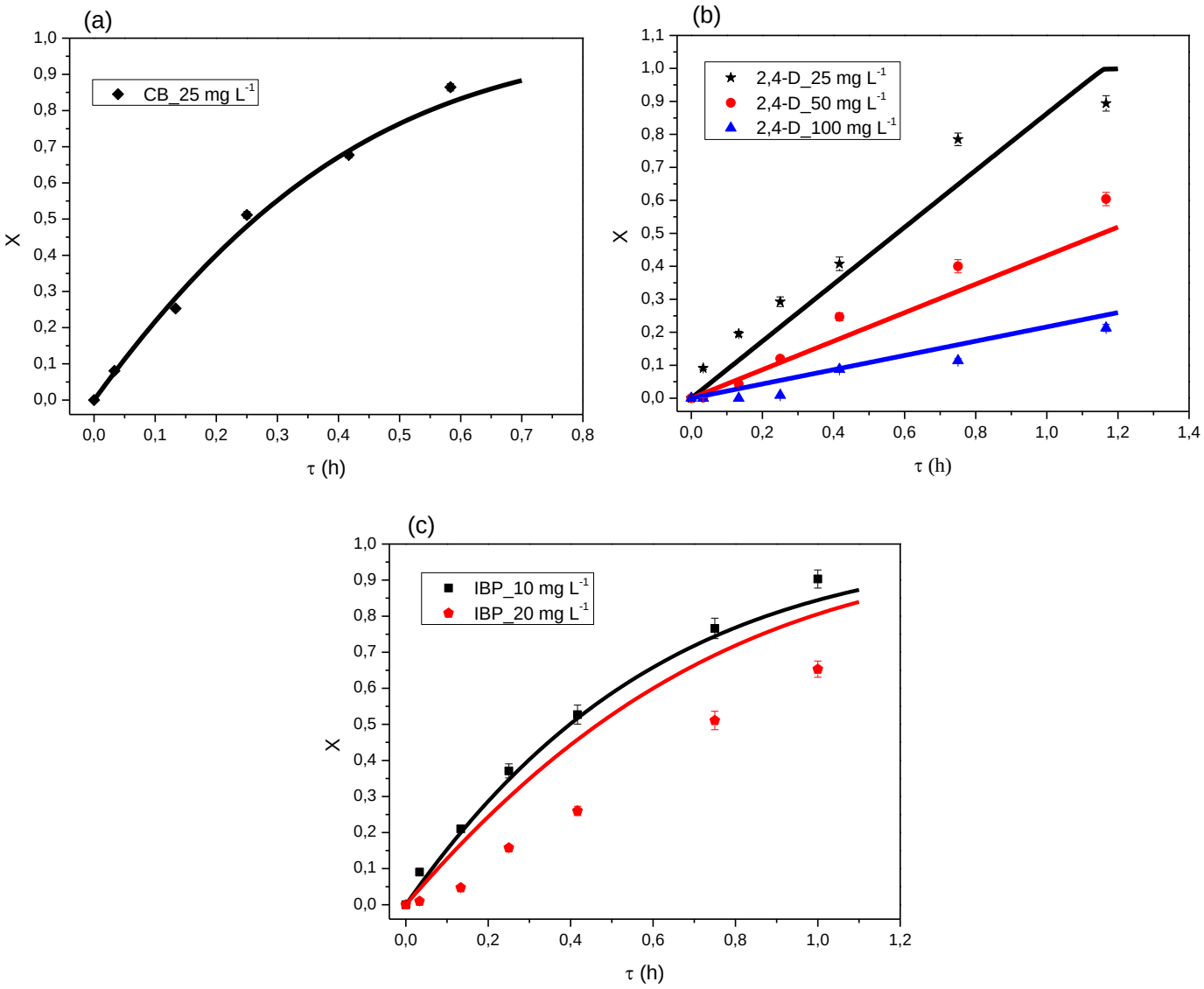

Figure 5.5. Kinetic modelling of CB (a), 2,4-D (b) and IBP (c) degradation in USPBRs: line presents the fitting to Michaelis-Menten model (T= 35 ºC).

Figures 5.5(b) and (c) show that the Michaelis-Menten model presents a notable variation of the experimental data at initial concentration of 20 and 100 mg L^{-1}; for IBP and 2,4-D, respectively; which indicates that these pollutants have concentration-dependent inhibition impacts for microorganisms in the bioreactor. Therefore, Michaelis-Menten model was extended with an

inhibitor factor (k_i) and the equation with 3 kinetic constants (equation 5.5) depict properly the degradation process (Wang & Loh 1999), distinctively of the initial concentrations (Figure 5.6).

$$r_P = -\frac{k_1 \cdot C_P}{k_2 + C_P + (C_P^2/k_i)} \qquad \text{(eq. 5.5)}$$

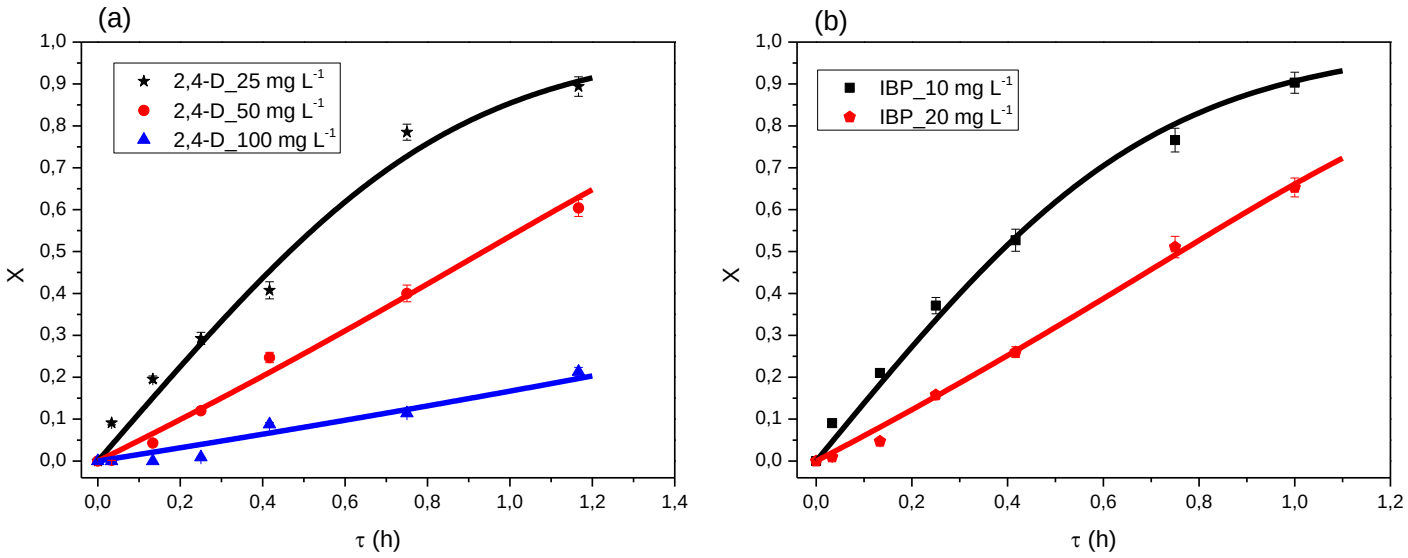

Figure 5.6. Substrate inhibition during 2,4-D and IBP degradation in USPBRs at the studied initial concentrations; continuous lines represent the fitting to the extended Michaelis–Menten model with the inhibitor factor.

Table 5.3 shows the kinetic parameters of the Michaelis-Menten with substrate inhibition model; the recalculated kinetic constants including the three initial concentrations (25, 50 and 100 mg L^{-1}) for 2,4-D and two (10 and 20 mg L^{-1}) for IBP, as can be seen, diverge of the previous ones; which may be associated with toxicity effects in the biomass at elevated initial concentrations, besides to have inhibition problems.

Table 5.3. Kinetic parameters of the Michaelis-Menten with substrate inhibition model for 2,4-D and IBP anaerobic biodegradation in USPBR.

Compound	k_1 (mmol g_{cat}^{-1} min^{-1})	k_2 (mmol L^{-1})	k_i(mmol L^{-1})	σ
2,4-D	0,55	0,19	0,071	0,02
IBP	0,35	0,11	0,019	0,001

5.5. Conclusions

The application of anaerobic USPBRs filled with BAC for the biodegradation of CB, 2,4-D and IBP in aqueous solution, resulted to be a very successful treatment; continuous experiments were achieved to assess the degradation capacity of the system.

The utilization of an slow agitation in the biological carbon bed derive in an increment of CB, IBP and 2,4-D, with a bioconversion up to 90% in space times below 0,6, 1 and 1,2 h, respectively. In addition, removal rates of 0,89 g L^{-1} d^{-1}, 0,46 g L^{-1} d^{-1} and 0,22 g L^{-1} d^{-1} were obtained for CB, 2,4-D and IBP, respectively. These results revealed that the degradation of these pollutants in this anaerobic configuration of bioreactor is an efficient and swift process.

The Michaelis–Menten model was found to describe the degradation process adequately for CB; however, IBP and 2,4-D presented a considerable inhibitory effect in the biofilm above initial concentrations of 20 and 100 mg L^{-1}, respectively. For this reason, a substrate inhibition factor was included in the Michaelis–Menten equation; the expanded model presented a well-fitting to the experimental data, regardless of the inlet concentration.

Chapter 6

Novel carbon materials as catalytic support for packed-bed reactors

6.1. Introduction

The removal of EPs like IBP using biological methods have demonstrated to be a cost-effective alternative and presents higher efficiency with notable outcomes over physico-chemical treatments. Nonetheless, and as mentioned in chapter 1 the reduction of several refractory contaminants occurs highly slowly, because of restrictions in the electron transfer rate. Thus, redox mediators appear to be employed as an electron bearer in many redox reactions; augmenting the reaction rate in one or more orders of magnitude (Pereira et al. 2013). The facility of handling the physical and chemical attributes of the carbon surface, the concentration and characteristics of the functional groups in the carbon materials are the main advantages of the redox mediators and therefore, make them suitable for the application as catalysts in the reductive transformation of hazardous contaminants (Amezquita-Garcia et al. 2015).

AC is widely known not only for their very good adsorbent and support properties, but also for the presence of a variety of surface functional groups that convert it in an effective redox mediator. In addition, it can be tailored to enhance its properties (Amezquita-Garcia et al. 2013; Pereira et al. 2010). Mezohegyi and coworkers (Mezohegyi et al. 2010) studied the anaerobic decolourisation of two textile azo dyes (Orange II and Reactive Black 5) in up-flow stirred packed-bed reactors with tailored activated carbons. The results evidence that the decolourisation rates were markedly swayed for the modified AC textural properties and slightly affected for its modified surface chemistry. Nevertheless, the use of carbon nanotubes (CNTs) and carbon xerogels (CXs)

as catalysts for recalcitrant compounds degradation is growing, due to their large mesoporosity (Soares et al. 2010). For example, CXs exhibit a high porosity, specific surface area and conductivity, manageable average pore size and can be prepared in the desired form (Mahata et al. 2007). CNTs have attracted the attention of the scientific community in the field of heterogeneous catalysis, due to their high surface areas, excellent electron conductivity, thermal stability and corrosion resistance (Liao et al. 2014).

The combination of these novel carbon based materials with packed-bed reactors appears to be a promising approach for the treatment of recalcitrant pollutants and may help speeding up the reaction rates. However, studies regarding the use of ACs, CNTs and CXs as catalysts support and redox mediator in anaerobic packed-bed reactors for the reduction of EPs are still lacking. Therefore, in this chapter the above mentioned catalysts were modified with chemical and thermal treatments for the purpose of obtain supports with different surface chemistries, in order to investigate in future works their possible influence on EPs degradation rates.

6.2. Characterization of carbon materials

6.2.1. Textural characteristics

The textural characterization of the different carbon materials treated in this study is compiled in table 6.1. As we can see, the treatments accomplished on

the original samples conduct to some changes in the textural characteristics of the carbon materials.

For original AC, we can see that is greatly microporous and its BET surface area is high (750 m^2 g^{-1}); whilst in the acid-treated sample (ACO) a slight decrease in the surface area (S_{BET}) is observed. This can be explained by the plentiful presence of oxygen-containing groups on the surface of the original AC that was introduced with the nitric acid (HNO_3) treatment and probably blocked the entrance of the N molecules within the pores (Messele et al. 2015). In addition, the micropore volumes (V_{micro}) and mesopore areas (S_{meso}) of AC and AC0 are different; suggesting that after the acid treatment the activated carbon mesostructure was partly destroyed (Messele et al. 2014). Moreover, a small reduction of the total pore volume (V_p) of ACO respect to AC was observed, while the pore size in both samples remained intact. On the other hand, the impregnation of samples AC and ACO with urea and melamine showed an appreciable reduction of the S_{BET}; it can be due to the existence of many groups which may block the access of N_2 to the tiny pores (Sousa et al. 2013). Nevertheless, a slight increase of the S_{meso} values was noticed on ACO_urea and ACO_mela compared to ACO, as expected; but a different trend was observed on samples AC_urea and AC_mela respected to AC. In addition, a decrease of V_{micro} was seen for all samples treated with nitrogen precursors. Usually, the textural characteristics of carbons are greatly affected by the nitrogen containing compounds and therefore also alter the micro, macro and meso porosity of the carbon materials. In the case of heat treated samples, ACO_400 (oxidized heat treated sample at 400 ºC) presented an increase of the S_{BET}, S_{meso} and V_{micro} comparing with the initial ACO, due to the elimination

of some oxygen-containing groups on the activated carbon surface during the thermal treatment (Tangsathitkulchai et al. 2009). On the contrary, samples ACO_600 and ACO_900 (oxidized heat treated samples at 600 and 900 ºC, respectively) showed a slight reduction in the S_{BET} value with respect to the initial ACO, which can be due to that the heat treatment was not applied efficiently for these samples and therefore the decomposition of the carboxylic groups and other groups, introduced with the oxidation treatment were not carried out completely (Figueiredo & Pereira 2010). However, the obtained results are within the experimental error provided by the equipment.

Regarding to the CNT samples, an increase of the S_{BET} was observed for CNTO when compared with the original CNT; this may be caused by the opening of the tips of the tubes (Monthioux et al. 2001). In the case of heat treated samples, similar behaviour was obtained; an increase of the S_{BET} was noticed after thermal treatment at different temperatures (400, 600 and 900 ºC). Several authors supported these results and justify it as a consequence of the removal of oxygen containing groups from the surface of the carbon nanotubes and therefore, increases access to carbon surface area for N_2 adsorption (Rocha et al. 2011; Gonçalves et al. 2010). Further, the CNT_urea sample also presented a small increase of the S_{BET} value, but the CNT_mela showed a reduction of the S_{BET} value; which can be explained by the high content of nitrogen groups that was introduced with the melamine on the imperfections of the CNT and consequently may obstruct the entrance of N_2 to the internal cavities. In addition, the existence of surface groups may play an important pulling effect between the nanotubes that force to bigger agglomeration of the

carbon material (Soares et al. 2015). On the other hand, a slight difference of V_p values was noticed for all studied samples and could be justified by the harshest oxidation conditions used in the preparation of the samples, that could attack the inner of the nanotubes (Gonçalves et al. 2010). The pore size (D_p) value remains constant for both samples. Furthermore, the carbon nanotubes present the minimum values and inexistence of micropores (Soares et al. 2010).

Table 6.1. Textural properties of the carbon materials.

Sample	S_{BET} ($m^2\ g^{-1}$)	S_{meso} ($m^2\ g^{-1}$)	V_{micro} ($cm^3\ g^{-1}$)	V_p ($cm^3\ g^{-1}$)	D_p (nm)
AC	750	242	0,27	0,47	0,77
AC_urea	728	226	0,27	0,45	0,81
AC_mela	558	188	0,20	0,36	0,74
ACO	725	125	0,28	0,41	0,77
ACO_400	786	209	0,26	0,43	0,84
ACO_600	677	204	0,26	0,43	0,77
ACO_900	708	211	0,27	0,44	0,77
ACO_mela	510	160	0,19	0,33	0,77
ACO_urea	688	207	0,25	0,43	0,84
CNT	273	273	0	0,57	8,01
CNT_urea	279	231	0,02	0,67	8,01
CNT_mela	222	222	0	0,51	8,01
CNTO	336	336	0	0,71	8,01
CNTO_400	361	361	0	0,75	8,01
CNTO_600	380	381	0	0,76	8,01
CNTO_900	379	379	0	0,81	8,01
CX_pH=5.3	592	144	0,21	0,39	0,77
CX_pH=6.9	572	290	0,15	0,49	1,81
CX5.3_urea	179	53	126	0,12	0,97
CX6.9_urea	475	204	270	0,70	0,74
CX5.3_mela	104	44	60	0,07	0,97
CX6.9_mela	482	113	369	0,41	0,74

Concerning to CXs, in the original samples (CX_pH=5.3 and CX_pH=6.9) a difference in the textural properties is noticed. Sousa and coworkers (Sousa et

al. 2012) conclude that these properties are affected in CXs, by the synthesis pH and the carbonization temperature. Although, the S_{BET} and V_{micro} are low in CX_pH=6.9 respect to CX_pH=5.3 and this behaviour is similar to those reported by (Sousa et al. 2012), a remarkable difference in S_{meso} is observed; it can be due to an experimental error during CX_pH=5.3 sample preparation and pH adjusting, affecting also the V_p and D_p characteristics of the sample. In addition, the S_{meso} is smaller than the activated carbon samples, which is a completely different behaviour to the obtained by (Soares et al. 2010). For CXs prepared with the nitrogen precursors at the studied pHs (CX5.3_urea, CX6.9_urea, CX5.3_mela and CX6.9_mela) diverse trends are observed. In the case of CX prepared with urea, an increment in S_{BET}, S_{meso} and V_{micro} is noticed, with increasing the pH; demonstrating an improvement of the porosity in CXs and that the reaction between formaldehyde, urea and resorcinol is advantageous. However, for CXs prepared with melamine, CX5.3_mela presents the lowest S_{BET}, S_{meso} and V_{micro}, respect to CX6.9_mela. The results are completely different to those obtained by (Sousa et al. 2012), where the CXs prepared with melamine at pH=5.3 and treated at various carbonization temperatures, exhibited the highest S_{BET}, S_{meso} and V_{micro}. This can be attributable to experimental errors during CX preparation and pH adjusting. Furthermore, CX6.9_mela shows the highest S_{BET},S_{meso} and V_{micro}, contradicting the affirmative statement that while increases the synthesis pH, the S_{BET} and V_{micro} of CXs prepared with melamine, decrease (Sousa et al. 2012).

6.2.2. Chemical characterization

6.2.2.1. Point of zero charge (pH_{pzc})

Table 6.2 show the values of pH_{pzc} for the prepared carbon materials. As can be seen, the oxidized samples (ACO, CNTO, ACO_400 and CNTO_400) present an acid character; which can be explained by the high quantity of oxygen surface groups inserted by the nitric acid treatment. However, the oxidized samples treated at high temperatures show neutral or basic characteristics, and this can be due to that after thermal treatments almost only CO groups are released on the carbon materials surface (Mezohegyi et al. 2010). In the case of samples treated with nitrogen precursors neutral or basic properties are observed, distinctively of the pH used in the preparation; it can be explained by the presence of nitrogen groups that provide basic characteristics (Messele et al. 2014; Sousa et al. 2012).

Table 6.2. Point of zero charge (pH_{pzc}) of the carbon materials.

Sample	pH_{pzc}
AC	7,5
AC_urea	8
AC_mela	8,4
ACO	2
ACO_400	2,3
ACO_600	7,5
ACO_900	8,5
ACO_mela	7,5
ACO_urea	7,3
CNT	7
CNT_urea	7,8
CNT_mela	7,5
CNTO	2
CNTO_400	4,3
CNTO_600	7
CNTO_900	8,3
XG_pH=5.3	7
XG_pH=6.9	8
XG5.3_urea	7
XG5.3_mela	7,5
XG6.9_urea	8,3
XG6.9_mela	8,8

6.2.2.2. Elemental and XPS analysis

Table 6.3 and 6.4 compiles the carbon (C), hydrogen (H), nitrogen (N), sulphur (S) and oxygen (O) contents obtained by elemental and XPS (C, N and O)

analysis, respectively. As can be seen according to elemental analysis (EA) results, for the original AC the nitrogen content is very low compared to the samples impregnated with the nitrogen precursors (AC_urea and AC_mela). Being an expected result, due to the largest quantity of nitrogen content was obtained in sample AC_mela, inasmuch as its molecule contains up to six atoms of nitrogen. In the case of the oxidized samples (ACO, ACO_urea and ACO_mela) an increase in the nitrogen content was observed respect to the original sample and the impregnated original samples (AC, AC_urea and AC_mela). This can be explained by the differences related with the nitrogen content present in the nitrogen precursors (67% in melamine and 47% in urea) and the intensifying effects of the surface acidity, augmented by the oxidation on the retention of N-containing organic bases (Bandosz & Ania 2006; Bagreev et al. 2004). Otherwise, for oxidized thermal treated samples (ACO_400, ACO_600 and ACO_900) decreased in nitrogen content was noted, that can be due to the removal of nitrogen-containing groups by increasing of temperatures. On the other hand, the oxygen content was calculated by the difference to 100 %. The results showed an increment in the oxygen content of the oxidized impregnated samples in comparison with the original ones, as expected. It seems that the presence of more oxygen groups have a leverage effect on inclusion of more nitrogen. Besides, with the oxidation treatment the oxygen groups can be reallocating with the trend to form more basic surface groups (Seredych et al. 2008). In the oxidized thermal samples a gradual decreased in oxygen content was observed, because of the removal of oxygen containing groups also at high temperatures. Moreover, a small increment of hydrogen

content was perceived afterward the treatment with HNO_3, possibly as a result of addition of acidic groups during the treatment.

Table 6.3. Elemental analysis of the original and modified carbon materials.

Sample	C_{EA} (wt %)	H_{EA} (wt %)	N_{EA} (wt %)	S_{EA} (wt %)	O_{EA}^{*} (wt %)
AC	85,9	1,26	0,12	0,55	12,2
AC_urea	85,2	1,38	0,72	0,52	12,2
AC_mela	80,2	1,43	4,22	0,42	13,4
ACO	73,5	1,80	0,60	0,49	23,6
ACO_400	84,2	1,70	0,42	0,49	13,2
ACO_600	85,4	2,03	0,46	0,48	11,6
ACO_900	88,5	1,97	0,47	0,45	8,62
ACO_mela	78,0	1,95	5,42	0,26	14,4
ACO_urea	80,9	1,90	1,09	0,46	15,6
CNT	97,8	0,42	0,01	0,05	1,67
CNT_urea	98,3	0,50	0,02	0,15	1,02
CNT_mela	91,4	0,60	5,31	0,14	2,57
CNTO	89,8	0,59	0,02	0,04	9,51
CNTO_400	94,8	0,33	0,01	0,03	4,83
CNTO_600	97,2	0,27	0,01	0,02	2,49
CNTO_900	97,2	0,27	0,02	0,02	2,47
XG_pH=5.3	87,1	2,63	0,00	0,06	10,2
XG_pH=6.9	90,8	2,59	0,00	0,01	6,57
XG5.3_urea	82,1	2,47	3,16	0,02	12,3
XG5.3_mela	84,3	2,81	4,77	0,06	8,10
XG6.9_urea	81,3	2,75	4,65	0,06	11,2
XG6.9_mela	79,8	2,54	4,51	0,03	13,2

*Oxygen determined by difference

In relation with CNTs, as can be appreciate a slight increment in nitrogen and hydrogen content was noted after the acid treatment or the functionalization with nitrogen precursors, respect to the original sample (CNT); this can be due to the inclusion of acidic and nitrogen groups during the respective treatments. However, a different trend was observed in CNT_mela which presents the highest content of nitrogen, due to the high amount of nitrogen atoms that contains the melamine in its structure, as was mentioned before. In addition, it is interesting to note that for the oxidized thermal treated samples (CNTO_400, CNTO_600 and CNTO_900) a small decreased in the hydrogen content was seen, which can be probably a consequence of the removal of hydrogen containing groups by the increment of temperature. Nevertheless, a huge increment of the oxygen content was noticed because of the formation of oxygen containing groups in the surface of CNTs, as result of the HNO_3 treatment.

Concerning to XGs, no nitrogen content was observed in the original samples at the different studied pH values (XG_pH=5.3 and XG_pH=6.9), similar results were obtained by (Sousa et al. 2012). But, a small difference in the hydrogen content was noted respect to the expected result that can be derivable from experimental errors during CX preparation and pH adjusting. Moreover, in samples prepared with melamine high nitrogen content was obtained respect to samples prepared with urea (irrespective of pH solution), as it was expected.

The XPS analysis gives information about the surface of the sample and its chemical composition, providing a better compression of the nature of the functional groups on the sample surface. Therefore, we decided to analyze by this technique those carbon materials rich in nitrogen groups and compare with the results obtained by EA.

Table 6.4 compiled the results obtained by XPS. As we can see for the AC and CX samples the nitrogen content decrease compare to the concentration of nitrogen determined by the EA. The results indicate that the concentration of N-groups is lower on the surface than in the bulk and the differences in the concentration of nitrogen could be associated with the nitrogen content present in urea and melamine (Seredych et al. 2008). However, it is interesting to mention that the loss of nitrogen groups is less in samples treated with urea, which can be due to that the nitrogen groups entered with this nitrogen precursor are more thermally stable than those inserted with melamine. For the CNTs samples treated with the nitrogen precursors no variation was observed in the nitrogen content, when compares the results of both analysis.

Table 6.4. XPS analysis for samples impregnated with nitrogen precursors.

Sample	C_{XPS} (wt %)	N_{XPS} (wt %)	O_{XPS} (wt %)	N6 groups (wt %)	N5 groups (wt %)
AC_urea	85,4	0,62	13,9	0,30	0,31
AC_mela	84,7	3,14	12,2	1,68	1,07
ACO_urea	78,8	2,99	18,2	1,53	0,95
ACO_mela	81,9	1,15	16,9	n.d.	0,99
CNT_urea	94,7	0,00	5,33	n.d	n.d.
CNT_mela	93,5	5,30	1,15	3,78	1,52
XG5.3_urea	89,3	3,85	6,85	2,11	1,22
XG5.3_mela	81,6	3,18	15,2	1,81	1,38
XG6.9_urea	90,7	4,64	4,63	2,48	2,16
XG6.9_mela	91,1	3,87	5,03	2,18	1,70

The quantification of the different nitrogen species present on the activated carbon surface was determined by deconvolution of the N1s XPS spectra. In all samples the common peaks were identified at 398 eV, corresponding to pyridinic-N groups (N6) and between 400-400,9 eV assigned to pyrrolic/pyridine-N groups (N5). As can be seen in AC and CNT samples the treatment with melamine nitrogen precursor contributes to major content of pyridinic groups and less pyrrolic/pyridine groups. Although, a little decrease in the content of pyrrolic/pyridine groups was observed in the pre-oxidized AC sample treated with melamine and a slight increase in the sample treated with urea, that can be the result of the differences in the chemistries of the interactions between nitrogen-containing precursors with the oxygen-containing

surface groups and in their pyrolisis mechanisms (Hulicova-Jurcakova et al. 2009). However, for the oxidized AC samples small quantities of quaternary nitrogen (NQ) group and forms of oxidized nitrogen (NX) were also observed at 401,4-401,7 eV and 402-403 eV, respectively. The oxidation treatment inserts 0,51wt % of NQ in ACO_urea sample and 0,16 wt % of NX in ACO_mela sample. Although, in AC_mela 0,39 wt % of NQ was observed, which can be due to the fact that at elevated carbonization temperatures N5 is transformed to N6 and consequently converted to NQ, indicating that during heat treatments chemical changes and transformation of the nitrogen groups occurs (Lahaye et al. 1999). Similar behaviour in CX samples was observed, higher content of pyridinic groups and less pyrrolic/pyridine groups were obtained. Nevertheless, in samples treated with urea there is more contribution of pyridinic groups than in samples treated with melamine, because in urea samples the nitrogen groups are found mainly on the surface and greater carbonization temperature pyrrolic/pyridine is transformed in pyridinic (Gorgulho et al. 2009; Sousa et al. 2012).

6.3. Conclusions

The surface chemical modification of three different carbon based materials was carried out, in view of their future use as redox mediator in anaerobic packed-bed reactors. An effective characterization of the carbon surface chemistry can help to identify the nature and concentration of the active sites.

The treatment with urea and melamine conduct to a severe reduction of the surface area, because of the presence of nitrogen groups on the carbon based materials surface and may partly obstruct the access of N_2 molecules to the micropores. However, a different trend is observed in samples treated with HNO_3, specifically with CNTs, in which an increase of the surface area was observed, and could be a result of the opening of the tips of the tubes. Although, for ACs samples thermally treated at high temperatures a decreased of the surface was observed, attributable to an inefficient heat treatment leading to the incomplete decomposition of the carboxylic and other groups inserted with the oxidation treatment.

In relation to the chemical properties, the point of zero charge of the studied catalysts diminish with the nitric acid treatment, while with the functionalization of nitrogen precursors and thermal treatments was noticed an increase.

Finally, in terms of nitrogen and oxygen contents an increase was observed in ACs and CNTs treated samples with nitrogen precursors and acid treatment, as a consequence of the incorporation of nitrogen and oxygen-containing groups during the corresponding treatments. Though, in heat treated samples a decrease in nitrogen and oxygen contents was seen, due to the removal of these groups by increased the temperature.

Chapter 7

Biodegradation of Acid Orange 7 in an anaerobic- aerobic sequential treatment system

7.1. Introduction

Several methods have been found to treat azo dye wastewaters, based on physical and chemical processes (Forgacs et al. 2004); among all the existing techniques, the most economic and environmentally friendly are biological treatments. However, because of the fact that azo dyes are artificial compounds and especially designed to be resistant in the natural environment, their biological degradation has serious obstacles. Investigations into the biodegradability of water-soluble azo dyes by an activated sludge process have indicated that, in most cases, these dyes could not be degraded under aerobic conditions, but the azo reduction can be relatively easy achieved under anaerobic conditions (Beydilli et al. 2000). In consequence, most of the products created by breaking of the N═N bond could be successfully degraded under aerobic conditions (Van der Zee & Villaverde 2005). Therefore, the major role of the aerobic stage is the reduction of both the non-dye and dye organic loading of the wastewater to the lower limits established by the environmental standards. Thus, these suggest a sequential anaerobic–aerobic process as the reasonable scheme for treating wastewaters containing azo dyes (Kalyuzhnyi & Sklyar 2000).

As it was mentioned in Chapter 1 (session 1.1) the removal efficient of AO7 using a continuous packed-bed type reactors in an up-flow mode filled with BAC was previously investigated in our research group; although, an improvement in the reactor system was required for the purpose to eliminate the excess of biomass in the bioreactor and make possible the kinetic modelling for the azo

dye decolourisation. The application of an appropriate agitation to the biofilm, resulted in an increment of AO 7 bioconversion up to 96 % at space time of 0,5 min (Mezohegyi et al., 2008). The obtained results demonstrated that the bio-decolourization in this system is higher to any other biological methods by at least one order of magnitude.

The employment of a special stirring in the biological bed can lead to losses of the BAC, which can flow out of the bioreactor in the outlet effluent and interfere in the quality of the obtained treated water. In addition, the obtained products of the azo reduction have been shown to be very bio-refractory; consequently, a separation system to confine the BAC and degrade the by-products is necessary. This step can be favourably conducted by aerobic membrane bioreactor systems (aerobic MBR), due to it can operate with very higher mixed liquor suspended solid concentrations, providing an improved biological treatment; and the membranes offer a barrier to solids. The two main advantages of the MBR treatment are their small design footprint and permeate (recovery water) of excellent quality (Bernal et al. 2002; Lahnsteiner et al. 2007).

Reduction of AO7 to SA and 1A2N by sewage effluent under anaerobic conditions has been widely reported in the literature but no a subsequent finishing biological treatment using aerobic MBR process. Hence, based on this observation the present chapter describes a sequential anaerobic-aerobic process for complete mineralization of AO7.

7.2. Anaerobic reduction of AO7 using a continuous USPBR

7.2.1. AO7/SA ratio

The reactor was fed with a constant concentration of AO7 (100 mg L^{-1}) for one month in order to encourage biomass acclimation to the azo dye. Under anaerobic conditions, the azo linkage in the reactive dyes was reduced and the large dye molecule was broken in two fragments. Thus, AO7 was degraded to SA and 1A2N, as shown in Chapter 3 (Figure 3.3). To confirm the proposed reaction and check if only the azo bond was broken in the dye molecule or whether there were subsequent reactions, SA concentration in the outlet was also determined. Figure 7.1 shows SA concentrations as a function of degraded AO7.

It can be appreciated that the amount of produced SA is proportional to the amount of decolorized azo dye in a ratio of 1:1, giving evidence that the proposed reaction takes place.

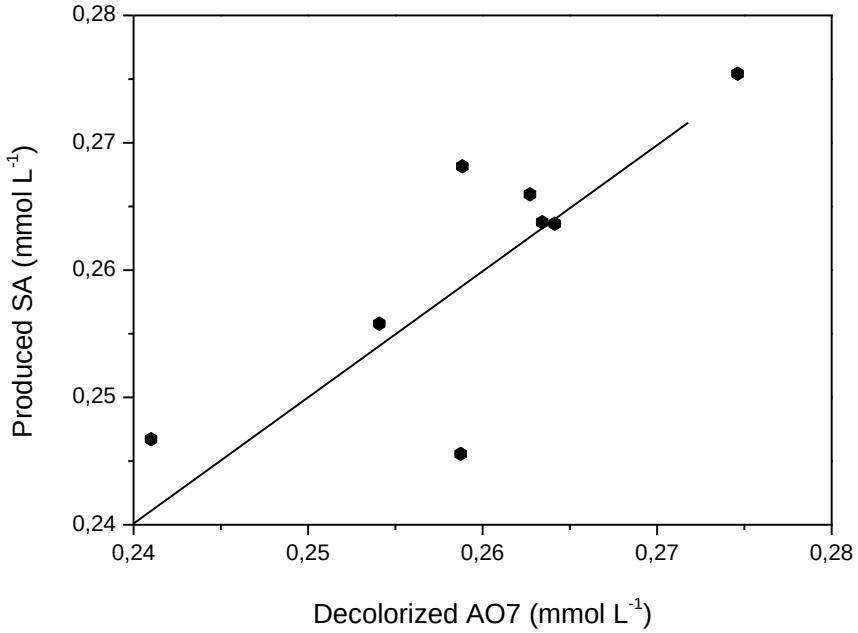

Figure 7.1. Ratio between destroyed AO7 and produced SA.

7.2.2. Continuous experiments

As previously mentioned in chapters 4 and 5 (sessions 4.2 and 5.3) is better to examine conversion values as a function of space time.

The continuous experiments were carried out in an anaerobic USPBR, where almost complete decolourization was achieved at short space time. AO7 conversion was about 99 % at space times of 1,02-1,6 min; these values of space times correspond to extremely short HRT of about 2,4 min and 4,4 min (with packed-bed porosity of 0,3), respectively. Results are shown in figure 7.2.

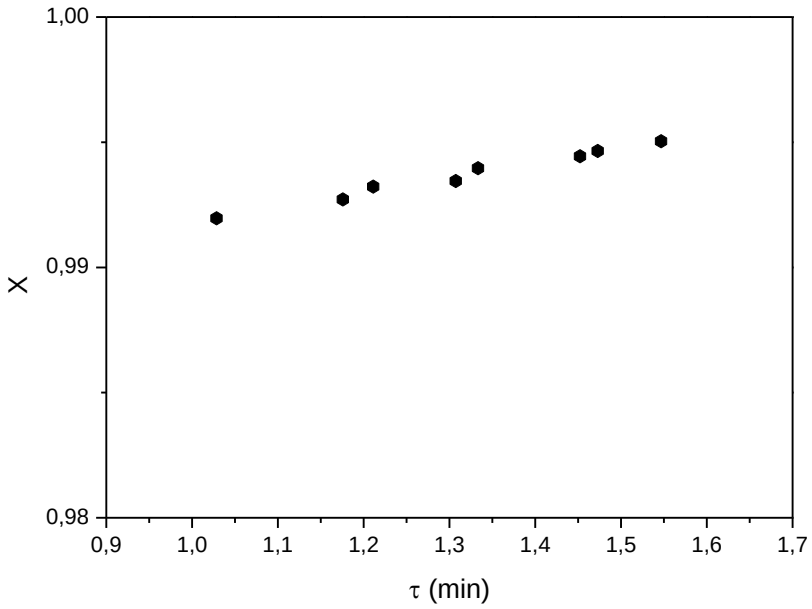

Figure 7.2. Acid Orange 7 conversion in continuous USPBR (⬣).

The obtained results were similar to those obtained by (Mezohegyi et al. 2007) for the same system, but using commercial activated carbon as catalytic support material.

7.2.3. Periodical stirring

After three months of operation at the USPBR slow but monotonous decreasing of dye conversion values was observed over the time. This can be explained by the isolation of metabolically active organisms on the sludge carbonaceous material surface by continuous expansion of biofilm around the catalyst (Scholz & Martin 1997). To avoid this problem, appropriate stirring of BSCM was applied in the packed-bed reactor. Stirring was first applied on day 95 at 1 h/day, and sampling was taking before the agitation of the biomass. Results are shown on

figure 7.3 and it can be clearly seen that azo dye conversion increased by applying stirring in the reactor.

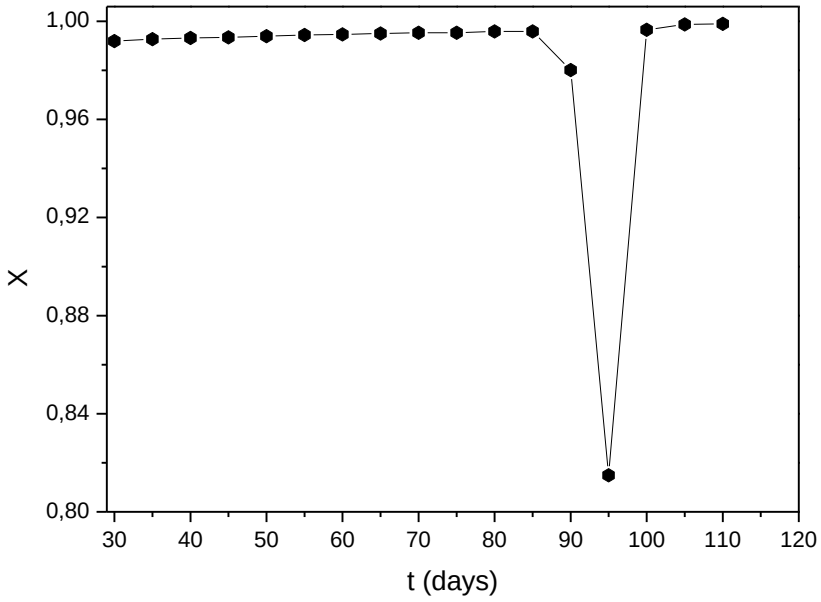

Figure 7.3. Effect of stirring in BSCM in the packed-bed reactor: (⬣) AO7 conversion.

Thus, the slow agitation of BSCM together with continuous flow of dye solution through the bed can help to remove the unnecessary amount of biomass from the reactor and keep a nearly constant concentration of microorganisms in the packed-bed; enhancing the performance of the SCM.

7.2.4. Aromatic amines

In all assays SA was not degraded and was accumulated in the medium in stoichiometric concentrations. This is the common behaviour of aromatic amines under anaerobic conditions, which are recalcitrant except for some few

aromatic amines substituted with hydroxyl and carboxyl groups (Tan et al. 1999). In the case of aromatic amines with sulphonic groups, their recalcitrant character is more marked (Leidner et al. 1980). On the other hand, there are different studies reporting successful SA degradation under aerobic conditions. Complete SA removal was achieved with an active sludge under aerobic conditions (Chen et al. 2012). Other researchers have reported the complete mineralization of SA using different types of aerobic inocula (Carvalho et al. 2008).

During the experiment a change in the anaerobic effluent was observed, the colour of the solution change from yellow to brown. This colour change was observed when samples were exposed to air. This fact occurs due to the presence of 1A2N the other metabolite, which is considered as a result of AO7 breakdown. However, it is very difficult to detect because its autoxidation in the presence of air occurs very fast (Méndez-Paz et al. 2005), even during the process of taking samples from the anaerobic unit. 1A2N oxidation yields 1,2-napthoquinone (12NQ) and was reported that 12NQ decomposes under aerobic conditions, which causes the change of colour of the solution (Keck et al. 1997). The reduction of this dye yields 1A2N, which is removed mainly to 12NQ and 2-naphthol (Kremer 1989).

7.3. Aerobic degradation of aromatic amines

7.3.1. Performance of aerobic MBR reactor at different HRTs

The COD and TOC remaining from the anaerobic reactor were mineralized under aerobic conditions in aerobic MBR reactor. The effects of HRT on the

COD removal efficiencies are shown in figure 7.4. It is observed that the COD removal efficiency was 17 % at a HRT of 8 h while almost 66 % of COD removal was achieved for a HRT of 48 h in the aerobic MBR unit, through the treatment of simulated wastewater. Although this COD removal efficiency was not too high comparing with the results obtained by (Işık & Sponza 2008) at almost the same HRT, it could be improved further by optimizing the parameters according to the actual conditions. However, in this stage the aromatic amines and the COD remaining from the USPBR reactor were readily degraded, mineralized and therefore, the brown colour present in the influent was removed. Furthermore the COD originated from the cleavage products of dyes was degraded. The data obtained in this study are comparable with the results obtained by (O'Neill 2000).

In addition, the use of a microfiltration membrane contributed for the organic matter removal, retaining colloidal organic compounds such as protein and polysaccharides generated from biomass decay (soluble microbial products), thus the membrane helps in the COD removal efficiency and avoid sludge washout.

TOC degradation in the influent and effluent of the aerobic MBR reactor were 64,38 and 29,38 mg L^{-1}, respectively; for a TOC removal efficiency was 54,37 %. The results obtained with this other method of analysis demonstrate a relatively significant fraction of TOC was removed by the microorganisms in the bioreactor. Therefore, coupled anaerobic–aerobic system has proven to be successful in achieving the complete biodegradation of AO7.

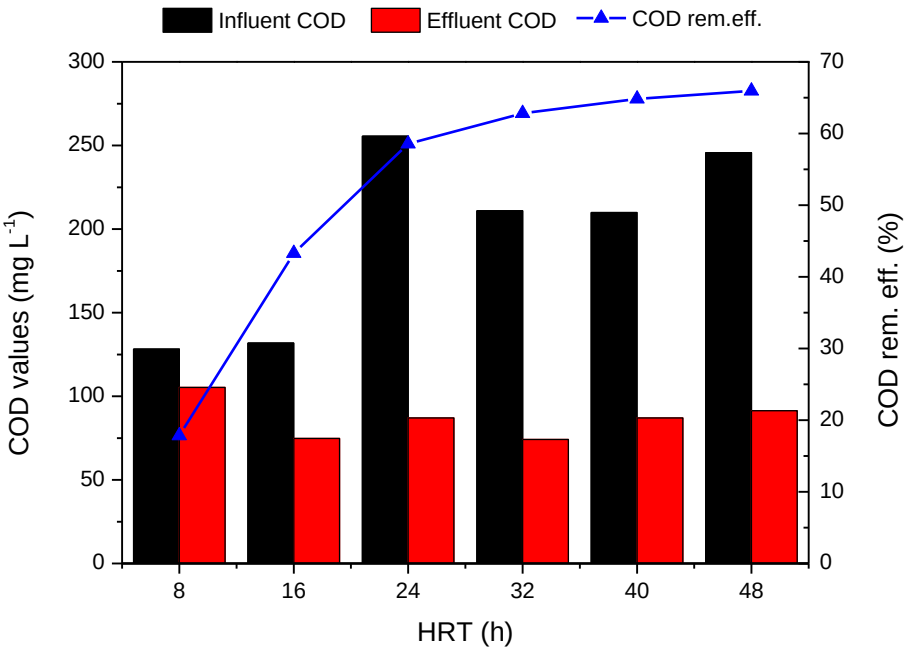

Figure 7.4. COD removal efficiencies in aerobic MBR reactor at different HRTs.

7.3.2. Total aromatic amine (TAA) removals in anaerobic/ aerobic sequential treatment

Figure 7.5 shows the aromatic amines and its removal efficiencies in an operation period, in order to see the removal efficiency of the whole system working in continuous. TAA concentration in the effluents of the USPBR/aerobic MBR reactor system was obtained from the SA levels produced from the chemical reduction of AO7 azo dye.

The primary reason for using an aerobic stage was to remove the amines. TAA values were found in a mean of 45,30 and 8,97 mg L^{-1} in USPBR and aerobic MBR respectively, during the studied period. This corresponded to a mean of 80,28 % of removal efficiencies in USPBR/aerobic MBR reactor system effluents. Similarly, Kalyuzhnyi and co-worker (Kalyuzhnyi & Sklyar 2000) found

that 56 % TAA removal efficiency was achieved in sequential anaerobic-aerobic hybrid reactor treating Sigrusgelb azo dye. Tan et al. (Tan et al. 2000) found that more than 50 % of SA is generated during Mordant Yellow 10 degradation with a mixture of anaerobic granular sludge and aerobic sludge.

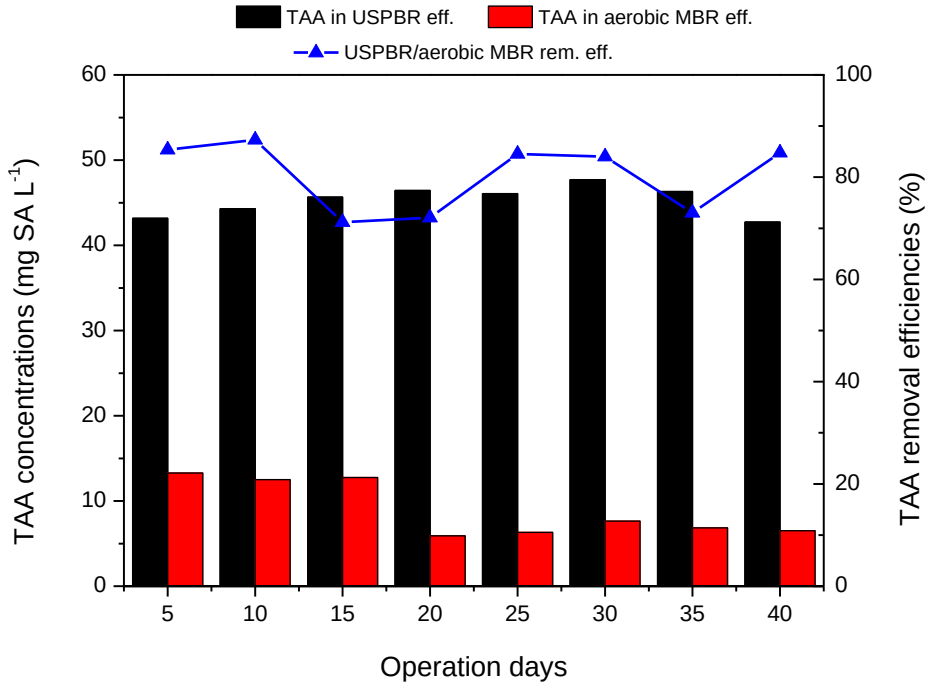

Figure 7.5. TAA values and TAA removal performance of USPBR/aerobic MBR in an operation period.

The results of this study showed that azo dyes were reduced to corresponding aromatic amines under anaerobic conditions which are resistant to further anaerobic degradation, however, they are well amenable for aerobic degradation. The complete mineralization of dyes therefore occurs via ultimate biodegradation under aerobic conditions.

7.4. Conclusions

The decolourization of azo dye AO7 was studied in a sequential anaerobic/aerobic reactor system; the anaerobic stage was carried out in a continuous USPBR filled with BSCM and aerobic stage took place in an aerobic MBR. In the continuous USPBR-BSCM system, azo dye bioconversion rates were about 99 % at very short space times (τ) 1,02-1,6 min. In the sequential aerobic stage, COD removal efficiencies were determined at different HRTs, obtaining the highest value about 66 % at 48 h. The TOC assay showed a removal efficiency about 54,37 %, suggesting the degradation of the aromatic amines produced in the anaerobic reactor. The TAA values were 45,30 and 8,97 mg L^{-1} in USPBR and aerobic MBR respectively, resulting in about 80,28 % removal efficiency. These results indicate that anaerobic sequential USPBR/aerobic MBR reactor system seems to be an effective and promising system for complete azo dye biodegradation.

Chapter 8

Conclusions and future work

8.1. General conclusions

Around the world, high volumes of wastewater are pumped straight in rivers, streams and the ocean every day. The severe impacts of this discharges into the environment, fisheries and to the human health itself, force the scientific community to enhance the actual wastewater treatments; in times where the water scarcity is also of much concern. Numerous treatment alternatives have been developed, however, bioreactor systems with immobilized microorganisms have proven to be sufficient and the most economical solution for wastewater remediation. Therefore, this thesis deals with the application of a previously technology developed in our research group, an anaerobic USPBR. The combination of the anaerobic and aerobic membrane bioreactor treatments has been studied to remove remaining bio-refractory compounds from the AO7 reduction. The preparation, surface chemistry modification and characterization of novel support carbon based material have been carried out in order to obtain redox mediators with good mechanical strength and high porosity, which are needed to achieve fast biodegradation rates in the bioreactor. AO7, nitrate, CB, 2,4-D and IBP were selected as model contaminants since they are considered priority risk pollutants, due to traces of them in water environments can affect the environmental health. The principal purpose of this research is to confirm the effectiveness of an innovative anaerobic packed-bed reactor for the production of high water quality from wastewater.

In general, the experimental results demonstrate the effectiveness of the USPBR and their promising application for the treatment of solutions containing

the selected pollutants. Continuous experiments carried out in USPBR filled with different biological carbon beds (BSCM or BAC) for nitrate removal under anaerobic conditions showed high nitrate removal efficiency, with values around 95-99%, at very short space times. In addition, as it was expected, the application of slow agitation in the biological packed-bed ensured high nitrate conversion at the same space time, demonstrating the positive effect of slow agitation of BSCM and BAC on nitrate removal. On the other hand, the denitrification process in USPBRs can be described by Michaelis-Menten model; the estimated kinetic parameters values correspond to the highest nitrate removal rates ever reported and it is evident that SCM might effectively compete with the commercial AC. Hence, the high efficiency and removal rate obtained in this study show that SCM played various roles, an excellent carrier material for the attachment of microorganisms and redox mediator for the enhanced nitrate bio-reduction in the USPBR.

For the biodegradation of CB, 2,4-D and IBP in USPBRs filled with BAC, the application of an slow agitation in the carbon bed played an important role in their bioconversion; assuring high conversion values (around 90 %) at short space times, below 0,6, 1 and 1,2 h, respectively. Moreover, the Michaelis-Menten model provided well-fitting to the experimental data of CB bioconversion, however, 2,4-D and IBP showed appreciable inhibition effects to the biomass at upper initial concentrations. Thus, a general model was assumed for the anaerobic degradation of 2,4-D and IBP in USPBR-BAC system, based on 2,4-D and IBP inhibition possible autocatalytic effects and the Michaelis-Menten kinetics. The high efficiency and removal rate obtained in this

study demonstrate once again, that the use of carbon support materials plays various roles, an excellent carrier material for the attachment of microorganisms and redox mediator for enhanced CB, 2,4-D and IBP bio-reduction in the USPBRs.

AC, CNTs and CXs were modified with dissimilar chemical and thermal treatments, including the functionalization with urea and melamine as nitrogen precursors and the liquid phase oxidation, with the objective to obtain carbon based materials with specific surface characteristics to meet the requirements of the expected catalytic reaction. The studied carbon materials were textural and surface chemistry characterized using various techniques, in order to determine their nature and concentration of functional groups. The oxidation treatment with nitric acid resulted in more acid carbon materials with high quantities of oxygen surface groups, but the following thermal treatments selectively eliminated those groups and help to increase the basic character of the samples. The treatment with the nitrogen precursors raise the surface basicity of the carbon materials and therefore, could intensify the interaction with acid molecules. Thus, the employment of these tailored carbon materials in the packed-bed reactor can achieve a substantial increment in the reduction rates of the studied recalcitrant contaminants.

Finally, the sequential anaerobic USPBR/aerobic MBR reactor system has proven to be successful in achieving the complete biodegradation and decolourization of AO7. The use of SCM as support material in the catalytic bed

of the USPBR has demonstrated again to be a good catalytic support; high conversion rates of AO7 were achieved at very short space times. The application of slow agitation in the carbon bed after certain period of the system working continuously, resulted in an increase of AO7 bioconversion. The released intermediates were mineralized in the aerobic part of the two stage system. It was observed that COD and aromatic amines were mainly degraded in the aerobic stage at low HRT. Thus, the TAA produced under anaerobic conditions was ultimately removed in the aerobic stage and the use of microfiltration membranes at the end of the process contributed to the organic matter removal.

8.2. Future work

Firstly, as future suggestions, the utilization of the novel and modified carbon based materials in the USPBR system for the anaerobic reduction of studied pollutants, is strongly recommended. The study and comparison of the influence of their dissimilar textural characteristics and diverse surface chemistries on pollutants reduction rates have to be conducted. Additionally, the adsorption capacity of the modified carbon materials and its saturation time has to be also investigated. In this context, the quality of the effluent, as well as the increase in the reaction rate, could provide accurate about the improvement in the performance of the packed-bed reactor with the tailored catalysts. Secondly, the aerobic membrane bioreactor unit has to be optimized in terms of HRT and better understand of membrane fouling.

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