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Organic Contaminants in Refinery Wastewater: Characterization and Novel Approaches for Biotreatment

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Abstract

Addressing major environmental issues, such as water pollution, is essential nowadays in realizing sustainable development. The ever-increasing world population and industrial development have led to the introduction of different types of chemicals to the environment, leading to considerable deterioration in environmental quality. A major class of these chemicals is phenolic compounds, which are hazardous pollutants and highly toxic even at low concentrations. In recent years, researchers have realized the importance of extracting new bacterial strains that are effective in treating different types of highly contaminated wastewaters at different severe conditions. They also focused considerable amount of research on developing new types of reactors that would provide efficient mixing and reduce mass transfer limitations. The aim is to develop and evaluate effective reactor systems and biocatalysts for the biodegradation of major contaminants in petroleum refinery wastewater. This chapter examines the different available options for the treatment of refinery wastewater with more focus on novel biotreatment options.

Keywords: biodegradation, wastewater, biotreatment, immobilization, phenols

1. Introduction

1.1. Pollution problem

Effluents from the chemical and petroleum industries contain many hazardous chemicals, which have resulted in the accumulation of severe environmental impacts. These effluents are rich in aromatic organic compounds such as polyaromatic hydrocarbons (PAHs) and phenolic substances that are barely degradable by nature; so they remain as a serious threat

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to the environment. They may accumulate in human and animal tissue upon long distance transportation. Some of these compounds like phenols are highly soluble in water and can be detected in wastewater in a wide range of concentration from a few milligrams per liter to as high as 7000 mg/l [1]. They may remain in water, and under favorable conditions, they can go through various reactions, such as chlorination and methylation, to produce even more harmful or more recalcitrant toxic materials like chlorophenols and cresols [1]. These organic compounds are among the most common forms of contaminants in industrial wastewater, and many of them show carcinogenic, teratogenic or mutagenic properties and were considered by the US Environmental Protection Agency EPA (USA) as priority pollutants. Thus, a great concern has been raised worldwide to remove these contaminants from industrial effluents before discharge into aqueous ecosystems, and it has been obligatory for industries to treat their wastewater effluents to ensure safe disposal to the environment.

Although collected efforts have been directed towards the replacement of fossil fuels, crude oil still keeps its place as a major source of energy and is expected to account for 32% of the world's energy supply by 2030 [2]. Refinery processes consume large amounts of water, and this makes them the main source of organic contaminants in wastewaters [3–5]. It was estimated that approximately 0.4–1.6 times the volume of the crude oil processed is generated as refinery wastewater (RWW) [2]. On the average, processing a barrel of crude oil consumes 65–90 gallons (246–341 l) of water [6]. Therefore, the oil industry will continue to discharge toxic waste into the marine environment. A decreased productivity of algae (a very important link in the food chain) was observed for water bodies receiving these effluents [2].

Because of a rising social and political concern on the environment, this water has to be properly treated to comply with the disposal limits imposed by the environmental legislations or to be reused [7]. The management of wastewater represents major economical and environmental challenges to most industries. Only a limited amount of work was directed to this topic for RWW. This chapter addresses the main concerns associated with the presence and treatment of organic contaminants in RWW with a focus on novel biotreatment approaches.

1.2. Sources of wastewater in petroleum refinery

Maximized benefits of crude oil are derived by processing crude oil in a refinery into a wide variety of products. More than 2500 refined products are produced, including liquefied petroleum gas, gasoline, kerosene, jet fuel, diesel fuel, lubricants, waxes and bitumen. Meanwhile, petrochemical industry derives its feedstock from refined products, which are transformed into valuable products such as plastics, synthetic materials and agro chemicals [4]. Large amounts of water are consumed in oil refineries for cooling systems, crude desalting, distillation, hydrotreating and during maintenance and shut down [8].

After initial fractionation, the crude passes through several treatment and conversion processes to reach the final blending stocks. Conversion processes include thermal and catalytic cracking, steam reforming, isomerization, alkylation and lube oil units, whereas treatment processes include naphtha and gas oil desulfurization, sour water strippers and catalyst regeneration units [8]. The composition of RWW is highly dependent on the complexity of the process. Al Zarooni and Elshorbagy [8] classified refineries as either hydroskimming or complex. A hydroskimming refinery comprises three sub units: a distillation unit in which

Unit	Wastewater major pollutants
Crude desalting	Free oil, ammonia, sulfides and suspended solids
Crude oil distillation	Sulfides, ammonia, phenols, oil, chloride, mercaptans
Thermal cracking	H_2S, ammonia, phenols
Catalytic cracking	Oil, sulfides, phenol, cyanide, ammonia
Hydrocracking	High in sulfides
Polymerization	Sulfides, mercaptans, ammonia
Alkylation	Spent caustic, oil, sulfides
Isomerization	Low level of phenols
Reforming	Sulfide
Hydrotreating	Ammonia, sulfides, phenol

Table 1. Major water sources in petroleum refining processes (adapted from [6]).

Parameter	Value range
pH	8.3–8.9
Conductivity (ms/cm)	5.2–6.8
Total suspended solid (mg/l)	30–40
Total dissolved solid (mg/l)	3800–6200
SO_4 (mg/l)	14.5–16
COD (mg/l)	3600–5300
Total phenol (mg/l)	160–185
Phenol (mg/l)	11–14
o-cresol (mg/l)	14–16.5
m, p-cresol (mg/l)	72–75
N-hexane (mg/l)	1.8–1.85
2,4- and 2,5-DCP (mg/l)	28–32

Table 2. Main characteristics of petroleum refinery wastewater (adapted from [6]).

crude oil is fractionated into various components, a reforming unit for reformate production and a desulfurization unit for reducing the sulfur content of some fractions such as kerosene and naphtha. A complex refinery incorporates a catalytic cracking unit additional to the hydroskimming refinery. Regardless of configuration, the waste effluent is the overall contribution of the units involved in crude oil processing. Al Zarooni and Elshorbagy [8] carried out an extensive program for the identification of major process and utilities wastewater streams and quantification of these streams relative to the total wastewater generated from all refinery processes.

1.3. Characteristics of refinery wastewater

RWW is characterized by a high chemical oxygen demand (COD) [5, 6], which results from the overall contribution of several aliphatic and aromatic hydrocarbons, emulsified oil and grease and inorganic substances, including ammonia, sulfides and cyanides [6, 9, 10]. Typical reported levels are 300–600 mg/l for COD, 20–200 mg/l for phenol, up to 3000 mg/l for oil and suspended solids of more than 100 mg/l [4, 8]. However, the quantity and characteristics of wastewater depend on the process configuration and complexity [4]. COD levels in the range of 3600–5300 mg/l were also reported [6]. **Table 1** summarizes the main pollutants in different petroleum refining units.

Typical analyses of effluents from a petroleum refinery are presented in **Table 2** as ranges of values. More data about the characteristics of RWW can be found elsewhere [2, 5, 8, 10–13].

2. Current treatment options

In line with the quest for a cleaner environment and bylaws of environmental compliance, removal of organic contaminants from RWW is a challenging task for achieving a sustainable development.

Al Zarooni and Elshorbagy [8] reported that dilution of the wastewater with process cooling water serves as the main approach applied to the RWW before disposal into the sea. It was strongly recommended to include primary as well as secondary treatment utilities to reduce the pollutant concentrations below the allowable standards for marine discharge.

Traditionally, there are two basic treatment stages. The first stage is a sequence of pretreatment steps, which employ mechanical and physicochemical techniques, whereas the second stage is an advanced treatment, which involves mainly a biological technique in the integrated activated sludge unit [2, 6]. The primary treatment is crucial for the efficient and prolonged performance of the secondary treatment unit. It targets the reduction of suspended matter like oil and grease to be achieved mechanically by gravity in separation tanks and then by a physiochemical step. In the advanced treatment, contaminants are decreased to specific acceptable discharge limits. In this regard, various solutions are proposed, including electrocoagulation, photocatalytic oxidation, wet oxidation, photodegradation, catalytic vacuum distillation, coagulation-flocculation, fenton oxidation, adsorption, microbial degradation, membrane bioreactor, ultrasonic degradation and chemical precipitation [5, 6].

Diya'uddeen et al. [2] and Rasalingam et al. [14] presented detailed reviews on the different treatment technologies for RWW, with a focus on photocatalytic degradation as an advanced oxidation process (AOP). AOPs have gained extensive attention due to the possibility of destroying a wide variety of organic substances by chemical oxidation, resulting in complete mineralization [2, 14]. In particular, heterogeneous photocatalysis has been demonstrated to be a promising efficient and cost-effective technique for RWW treatment at an advanced stage. However, the industrial application of this technique is greatly limited by the scarcity of available information in the literature [2]. Remya and Lin [15] reviewed the current status

of microwave application in wastewater treatment. The following sections will present a brief summary of the main options and highlight the main benefits and drawbacks of each option.
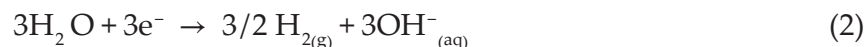
2.1. Electrocoagulation

Electrochemical technology has attracted great attention in recent years for wastewater treatment for the many featured advantages such as environmental compatibility, versatility, energy efficiency, safety, easy automation, selectivity and cost effectiveness [3, 5]. Traditional electrochemical methods include electrocoagulation, electroflotation, electroflocculation, electrochemical reduction and electrochlorination. Electrocoagulation (EC) is based on utilizing "sacrificed" anode, which corrodes by an applied electrical current, for in situ formation of a coagulant. Small dispersed particles combine into larger agglomerates which can be removed by precipitation, floatation or filtration. EC is efficient for removing suspended solids, oil and greases. The reactions occurring in an electrochemical cell with aluminum electrodes are as follows [9, 13]:

At the anode:

$$Al_{(s)} \rightarrow Al^{3+}_{(aq)} + 3e^- \tag{1}$$

At the cathode:

$$3H_2O + 3e^- \rightarrow 3/2\, H_{2(g)} + 3OH^-_{(aq)} \tag{2}$$

In the solution:

$$Al^{3+}_{(aq)} + 3H_2O \rightarrow Al(OH)_{3(s)} + 3H^+_{(aq)} \tag{3}$$

To explain the theory, EC occurs through a sequence of steps as (i) electrolytic reactions at electrode surfaces, where Al ions form at the anode and hydroxyl ions are generated at the cathode, (ii) in situ oxidation of Al ions followed by precipitation of aluminum hydroxide in aqueous phase and (iii) adsorption of soluble or colloidal contaminants on coagulants which are removed by sedimentation or flotation. Electrode material has a crucial role in the mechanism of electrocoagulation, which is also highly influenced by the chemistry of the aqueous medium, especially conductivity [4]. The performance of the electrochemical system is highly affected by the initial composition of the wastewater and the current density [9]. Treatment efficiency, in terms of energy and electrode consumption, is affected by the operating conditions [13].

In spite of the attraction of electrocoagulation, its application for real RWW is rather scarce and limited in the literature. Nevertheless, real application and related performance and design considerations have been addressed by a few studies [4, 5, 9, 12, 13].

2.2. Activated carbon adsorption

Adsorption using activated carbon has frequently been considered the most efficient technique for removing nondegradable waste pollutants, with a high adsorption capacity, flexibility

and simplicity of design, low operational cost and the advantage that activated carbon can be regenerated and reused. During the adsorption, phenol is not degraded but rather transferred from the RWW to another phase, which results in the formation of serious by-products (secondary pollution). As such, regeneration is essential for reusing the activated carbon and for the collection and reuse of the contaminant substance [14, 16]. Activated carbon is characterized by a wide variety of pores. The adsorption process proceeds through a sequence of diffusion steps from the bulk phase into the micropores, which are thought to be the major adsorption sites on activated carbon. Also, the amount adsorbed was reported to be positively influenced by increased temperatures and decreased particle size of the adsorbent [14]. In adsorption, adsorbate-adsorbent interaction plays a key role. The lack of such effective interaction results in only moderate adsorption capacity [14]. Adsorption isotherms are established by estimating uptake (or equilibrium adsorption capacity), q, calculated from the difference between the initial and the final phenol concentrations as follows:

$$q = \frac{(C_i - C_f)V}{m} \qquad (4)$$

where q is the uptake (mg/g), C_i and C_f (mg/l) are the pollutant initial and final concentrations, respectively, m is the adsorbent dosage (g) and V is the solution volume.

A wide variety of equilibrium isotherm models have been developed. However, the Langmuir isotherm and the Freundlich isotherm are the most commonly used. The former has been applied to a variety of pollutant processes, which involve homogeneous surfaces and negligible interaction between the adsorbed molecules. A main assumption is an arrangement of monolayer adsorption on the adsorptive sites. On the other hand, the Freundlich isotherm is applied for nonideal and multilayer adsorption processes [14].

The high cost of commercial activated carbon has drawn attention to other alternatives. El-Naas et al. [16] evaluated the effectiveness of activated carbon locally prepared from date-pits (DP-AC) for the removal of phenol from RWW. The results proved DP-AC to be a promising low-cost alternative to commercial activated carbon. Furthermore, different approaches were tested for the regeneration of saturated activated carbon and 86% regeneration efficiency could be achieved after four regeneration cycles. Effective utilization of DP-AC was also proved for the reduction of COD in refinery wastewater [17].

2.3. Biological processes

The conventional activated sludge process has been widely used for the removal of organic contaminants from RWW, in an integrated system that includes mechanical and physio-chemical pretreatment [6]. Biological means are incapable of completely removing recalcitrant organic material usually encountered in RWW. These obstacles may be tackled by bioaugmentation, which involves the introduction of robust indigenous or genetically modified organisms. However, a major drawback of bioaugmentation is the uncertainty of reproducibility when the process is transferred to a full scale due to the effects of many variables [2]. To enhance biodegradability, biofilm reactors have been proposed as they prove to be more efficient than conventional biological systems. Biological processes have

been identified as suitable and cost-effective method for wastewater treatment. However, conventional processes suffer from many different operational problems such as inhibition at high concentration of toxic substances, long retention time and/or start-up periods, weak tolerance to shock loads and excessive sludge formation [10]. Bacterial activity can be enhanced in immobilized cell reactors, which offer many advantages over suspended cell reactors as will be explained later [18].

Substitution of the conventional activated sludge process continues to be an attractive topic of research. In this regard, Viero et al. [10] treated RWW in a submerged membrane bioreactor as a robust process that allows operation under shock loading rates and hydraulic fluctuations. This is mainly a combined process that utilizes a bioreactor and a granulated activated carbon filter. Also great attention has been directed to anaerobic-aerobic treatments. Chan et al. [19] provided a detailed review of the various types of anaerobic-aerobic wastewater treatment techniques including the new technologies aimed at developing high rate bioreactors and integrated anaerobic-aerobic bioreactors. However, most of these integrated bioreactors lack information on industrial implementation.

2.4. Combined or integrated methods

Complete degradation of persistent organics like chlorinated phenols through biological means proves to be difficult due to the biorefractory nature of these compounds. So, there still exists a need for advanced schemes and devised combinations of treatments for the complete removal of such contaminants. Pretreatment technologies are very effective in decreasing the priority pollutants concentration before the biodegradation step. Several solutions are proposed including the use of coagulants and electrochemical oxidation, Fenton oxidation and ozonation [6]. Combined photochemical or electrochemical pretreatment and biological processes are well documented [6, 20]. In addition, the effective combination of adsorption and biodegradation processes has long been approached by many researchers [6, 21]. For example, ozonation was combined as a pretreatment step with biodegradation for the decomposition of chlorinated phenols. Ozonation is a chemical oxidation method and its combination with a bioprocess results in less toxic compounds, thus enhancing the overall process efficiency and reducing the treatment time and cost. A removal efficiency of 85% could be achieved for 4 chlorophenol (4 CP) that initially could not be degraded in a pure biological step, whereas the degradation of 2,4-dichlorophenol (2,4-DCP) was improved from 40 to 87%. However, several issues were raised as to the high cost and lack of information on technical aspects of the process [22].

The combination of AOP as chemical pretreatment and biological processes was found particularly useful in enhancing the biodegradability and was recommended as a successful technology for industrial wastewater treatment. In this context, Fenton oxidation and/or reductive dehalogenation were used as a pretreatment of chlorinated aromatic compounds before the start of the biological process [23]. The combination of microwave irradiation with AOPs has also been discussed in detail [15]. The choice of a pretreatment method cannot be generalized as it depends upon several factors, including the type of contaminants, real conditions and process costs [23].

3. Advantages and drawbacks of current options

Many of the aforementioned methods have been recognized as efficient techniques for the treatment of RWW and to offer a lot of featured advantages such as energy efficiency, safety and environmental compatibility [3, 13]. However, most of these physiochemical methods suffer from noticeable drawbacks such as high capital and operating costs. Ultimately, most of them do not destroy the contaminant, but rather transfer it to another phase, which results in the formation of harmful by-products [13, 16]. Formation of chlorinated organic compounds has been reported during some electrochemical applications, and activated carbon adsorption was recommended as a further polishing treatment to remove them [5, 12]. Also formation of intermediate by-products like catechol and hydroquinone was detected after ozonation of phenolic compounds [22]. Moreover, most of these methods are incapable of treating heavily contaminated water with COD levels above 4000 mg/l [5].

Although AOPs have emerged as effective methods, which offer a chance for the mineralization of various biorefractory organics [14], the running cost of many AOPs is still relatively high [15]. Also, they were reported for the formation of by-products and to be limited to treating wastewater with relatively low COD concentration [24]. Many other methods which are relatively cheap and easy to operate are characterized by strict technical limitations, in terms of operating conditions and effluent hydraulic rates (e.g. Fenton and membrane applications), low efficiencies and excessive sludge generation (e.g. membrane applications) or in terms of high energy consumption like microwave effects, which cannot be utilized by conventional heating [2, 15]. On the other hand, some applications are limited by the hazards associated with them like in ozone utilization, being an unstable gas [2]. Others are difficult to be commercialized for real-time RWW treatment, and large-scale industrial applications seem to be lacking.

In view of the above discussion, it is essential to search for more viable alternatives that can be utilized in novel biological treatment systems. As for the several mixed processes that have been proposed recently, a lot of these treatment schemes not only have noticeable advantages but also have important drawbacks. These problems can be sorted in two main areas: the first relates to all economic aspects including the high cost needed for the implementation of these techniques; the second includes all technical issues related to the resources needed for the transformation from very toxic compounds to environmentally compatible ones [22].

4. Why is biodegradation favorable?

Biodegradation is the decomposition of organic substances by microorganisms into metabolic by-products with lower toxicity. Enzymes play a catalytic role in this process, where a chemical is converted stepwise into end products through various intermediates. This transformation is called mineralization [25]. Biodegradation is a cost effective and environmentally compatible option that is often preferred, thanks to the possibility of complete mineralization [26, 27]. Because of the aromatic structure of many organic compounds (e.g. phenols),

they are highly stable due to the difficulty of cleaving the benzene ring. However, several microorganisms have the capability to utilize these compounds for their metabolic activities as carbon and energy sources. Biological transformation has been recognized as one of the key solutions to deal with environmental pollution caused by many problematic organic contaminants. In this regard, the use of pure and mixed cultures of organisms is considered a favorable and most promising approach [28]. Many strains of bacteria, fungi and algae have the ability to degrade toxic organic substances. Bacterial cultures of *Pseudomonas* genus are the most commonly utilized biomass for the biodegradation of organic contaminants, with special interest paid to *Pseudomonas putida* due to its high removal efficiency [29]. However, a main drawback in bioprocesses is the inhibition of the enzymatic activity at high substrate concentrations. Under certain conditions, organic material can be decomposed aerobically or anaerobically [30]. Conventionally, aerobic processes are preferred. Aerobic microorganisms grow faster and are more efficient because they can achieve complete mineralization of toxic organic substances to inorganic constituents (CO_2, H_2O) [31]. This is in addition to low associated costs [16]. On the other hand, the end products of biochemical reactions in anaerobic processes often produce esthetically displeasing colors and fouling odors in water [2]. Therefore, there is a limited interest in the utilization of anaerobic microorganism for the degradation of organic waste. However, there have been several studies in this regard [32–34]. Since most biological treatment studies have used aerobic biomasses, discussion in the following sections of this chapter will focus on aerobic biodegradation. Detailed reviews on the biodegradation of some organic compounds can be found in the literature [27, 35–37].

4.1. Mechanisms of biodegradation

Biodegradation is a multivariable process, which is affected by a combination of many biotic and abiotic factors, including pH, temperature, oxygen content and availability, microbial abundance and substrate concentration [26, 27]. The chemical structure of aromatic compounds plays a key role as reflected by the number, type and position of substituents on the aromatic ring and degree of branching. The greater the number of substituents in the structure, the more toxic and less degradable it is.

Metabolic processes are dominated by the catalysis of enzymes, which are particular to each type of biomass and reaction. A metabolic reaction is ultimately a process of energy conversion. Little is known about the biodegradation mechanism by fungi and algae; so the following is a brief discussion of this mechanism by aerobic bacteria, as typically represented by the biodegradation of aromatics.

In aerobic biodegradation, enzymatic attack on the aromatic ring is initiated by oxygen. A typical pathway for metabolizing phenols (phenol is a basic structural unit for a variety of synthetic organic compounds) is to hydroxylate the ring by the enzyme phenol hydroxylase, form catechol and then open the ring through *ortho-* or *meta*-oxidation. Thus, phenol hydroxylase is the first enzyme and catechol is a basic intermediate in the degradation pathways of many aromatic substances. In the *ortho*-pathway, the aromatic ring is cleaved by the enzyme catechol 1,2-dioxygenase (C12O). In the *meta*-pathway, the ring is cleaved by the enzyme catechol 2,3-dioxygenase (C23O). The ring is thus opened and then degraded [27].

(C12O) and (C23O) designate two different orientations as to how the ring cleavage can occur. However, the biodegradation of many aromatics proceeds through the *ortho*-cleavage pathway after the formation of catechol because the *meta*-cleavage results in the formation of dead end metabolites from catechol; the enzyme gets inactivated by the accumulation of a toxic intermediate [38]. As a rule, conversion of catechol does not follow the *meta*-cleavage pathway, and generally, the *ortho*-cleavage pathway is required for the complete degradation of many aromatic organics [39]. Discussion of biodegradation pathways and mechanisms can be found in [27, 35, 36, 40].

4.2. Biodegradation kinetics and modeling

Kinetic studies indicate how effectively a bioprocess is functioning. This is essential to improve process control and removal efficiency [31]. The main step in modeling a biodegradation process is to relate the specific growth rate of the biomass to the consumption rate of the substrate [26]. Different kinetic models have been used to describe the dynamics of microbial growth on phenols [35].

Based on material balance, the rate of biomass growth and the rate of substrate utilization (both in mg/l h) can be represented by Eqs. (5) and (6), respectively:

$$\frac{dX}{dt} = \mu X - k_d X = \mu_{net} X \left(\text{or } \frac{d\ln X}{dt} = \mu_{net} \right) \tag{5}$$

$$\frac{dS}{dt} = -\frac{\mu X}{Y} \tag{6}$$

where Y is the cell mass yield (g/g) = dX/dS; X is the biomass concentration (mg/l); S is the substrate concentration (mg/l); k_d is the decay coefficient (h^{-1}) (often ignored) and μ is the specific growth rate (h^{-1}).

There are two most common models for cell growth during the biodegradation: the Monod model and the Haldane model, represented by Eqs. (7) and (8), respectively:

$$\mu = \frac{\mu_{max} S}{K_s + S} \tag{7}$$

$$\mu = \frac{\mu_{max} S}{K_s + S + \left(\frac{S^2}{K_i}\right)} \tag{8}$$

where K_s (mg/l) is the half saturation coefficient (related to microorganism's affinity to the substrate) and K_i is the substrate inhibition constant (mg/l). The first model neglects the substance inhibition, whereas the second model is the most widely used since it accounts for the inhibitory effect of toxic material and it is mathematically simple [26, 41]. It is noteworthy that when K_i is very large, the Haldane equation reduces to the Monod model.

Although cell immobilization by entrapment offers significant advantages, it is problematic in terms of diffusion limitation owing to the resistance imposed by the protective structure [42]. Also, in the design and modeling of bioreactors, it is equally important to assess external mass transfer coefficients for the transfer of substrate from the bulk phase to the surface of

the biofilm. Diffusion limitations considerably affect the intrinsic reaction kinetics. Therefore, kinetic models for degradation in biofilm reactors are complex and difficult to develop. Several studies addressed this topic and proposed comprehensive diffusion-reaction models, which account for mass transfer limitations [42, 43].

5. Novel approaches in biotreatment

In light of the fact that biodegradation of aromatic organic pollutants has a unique environmental and ecological impact, there is a rising interest in this area of biotechnology and a strong motivation to develop efficient strategies and novel cost-effective methods for RWW management. This section will briefly review some of the achievements on this track.

5.1. Immobilization techniques

Immobilization is an effective technique that is usually used for many purposes, mainly the protection of the biomass from toxicity effects of the contaminant, in addition to the ease of separation and reutilization of the biomass [35]. Other advantages were highlighted in the previous section. Bacterial activity can be increased by various immobilization methods like bead entrapment, carrier binding, encapsulation, cell coating and film attachment [44].

Polyvinyl alcohol (PVA) is a synthetic polymer that shows attractive properties to be used as a carrier in bacterial immobilization; it is inexpensive, nontoxic, water soluble and has excellent electrical insulation [45]. El-Naas et al. [46] evaluated the characteristics of PVA gel matrices with immobilized *P. putida*, prepared by crosslinking in the gel structure by repeated cycles of freezing-thawing, resulting in a highly porous fibril structure with high mechanical strength and elastic rubbery nature. The effectiveness of these gel pellets with immobilized *P. putida* was investigated for the biodegradation of phenol in different reactor configurations, in batch and continuous modes, and proved to be effective even at high phenol concentrations [26, 29, 47, 48]. Emphasis was placed on the contaminant uptake per mass of PVA, which is of particular economical importance in the design of RWW treatment processes. Compared to natural biodegradable Ca-alginate, which has long been used in the area of biocatalysts, PVA gel is more durable with lower resistance to mass transfer due to its porous structure.

In view of that fact that biodegradation is in essence a series of enzyme-catalyzed reactions, there has been an increasing attention to the direct application of immobilized robust enzymes obtained from degrading microorganisms for the treatment of contaminated wastewater. Enzymatic treatment of organic pollutants is documented in a few recent studies, which addressed novel, practical and inexpensive immobilization methods. Extensive discussion on this topic can be found in a review by Demarche et al. [49].

Attention in recent years has focused on developing aerobic granules in sequencing batch reactors (SBRs) [50]. This is a novel biotechnique, characterized by self-immobilization of microorganisms through cell-to-cell adhesion, without any carrier material. Aerobic granules are reported for a very high cell concentration (up to 15,000 mg/l) and to be able to

decontaminate high-strength wastewater (up to 15 kg COD/m^3/day) [51]. However, this technology is still in a development stage. Detailed information on aerobic granulation can be found in a review by Khan et al. [50].

5.2. Utilization of spouted bed bioreactor (SBBR)

The SBBR is superior to the conventional bubble column bioreactor with its characteristic advantage of efficient intense mixing that is induced by cyclic motion of particles within the bed. The cyclic motion of particles results from a single air jet injected through an orifice in the bottom of the reactor [29, 46–48]. This reactor design offers better contact between substrate and cells as well as faster oxygen and nutrient transfer rate, which leads to higher removal rates of the contaminant. Details about the SBBR can be found elsewhere [32]. El-Naas et al. [29] proved that the efficient mixing in the SBBR has a significant role in overcoming the external mass resistance for the transfer of substrate from the bulk phase to the surface of the biofilm, and thus uniform concentration across the height of the reactor is a justified assumption. Effective utilization of the SBBR for the biodegradation of phenol and its derivatives and for the treatment of RWW is reported in several studies as will be illustrated in the next section.

5.3. Different applications

Example applications of novel biotreatment approaches for RWW treatment are briefly discussed in the following sections.

5.3.1. Biodegradation of phenol

Owing to the diversity of RWW composition, phenols have been adopted as a suitable measure for the performance of biodegradation [10]. *P. putida*, immobilized in PVA gel particles, has been successfully utilized for the removal of phenol from simulated wastewater, and the immobilized bacteria proved to be resilient in sustaining deprivation of nutrients and sudden exposure to high concentrations of phenol [48]. Batch and continuous biodegradation of phenol by PVA-immobilized *P. putida*, in bubble column reactor and a specially designed SBBR, was investigated in several studies and assessed for the effects of various variables. SBBR was shown to have superior performance. The biodegradation rate, being highly dependent on temperature, pH and initial phenol concentration, was optimized at 30°C, 7 and 75 mg/l, respectively. The experimental data fitted better to the Haldane inhibitory model (Eq. (8)) than the Monod model (Eq. (7)). However, the inhibition effect was not encountered in the continuous operation up to an initial phenol concentration of 150 mg/l, thanks to the continuous dilution effect. A mathematical dynamic model that incorporates the effect of internal mass transfer resistance and growth kinetics in the SBBR as represented by the Haldane model was proposed. The experimental data fitted fairly well to the model; the dynamics of the system is mainly controlled by the mass transfer [47]. The biodegradation rate also depends on biomass abundance as reflected by a linear increase in the biodegradation rate with the amount of PVA gel, the phenol uptake per mass of PVA reached a maximum at a PVA volume of 10 ml within a total volume of 1 liter; more PVA particles in the bioreactor is expected to have adverse effect

by hindering particle movement and mixing [47]. The importance of the uptake value in the design of industrial RWW treatment processes was emphasized in Section 5.1.

It is a matter of fact that mass transfer limitations have significant effect on the biodegradation rate. Two other important parameters, which are directly related to the mass transfer limitations, are the PVA particle size and the air flow rate [26, 29]. Mass transfer is directly related to the accessibility of the biomass to phenol; it was enhanced by decreasing the PVA particle size and increasing the air flow rate. This effect was more pronounced for high air flow rates and low phenol concentrations. This could be due to improvements in mass transfer induced by the combined effect of good mixing and reduced particle size. The effect of the air flow rate was closely examined; the biodegradation rate increased linearly as the air flow rate was increased from 1 to 3 l/min; then, it became almost constant. In this regard, two main factors are believed to be involved: mixing and aeration. The effects of these two factors were assessed separately, and it was shown that at air flow rates higher than 1 l/min, the main one of the two factors is the availability of enough oxygen to complete the biodegradation reaction. However, the effect of mixing may be more significant for smaller PVA particle size or very low initial phenol concentrations. Therefore, the external diffusion plays a dominant role as a controlling mechanism at low bulk concentrations. The intense mixing that is characteristic of the SBBR proves to be effective in overcoming these mass transfer limitations, which makes it an efficient reactor for the biodegradation of phenol.

5.3.2. Biodegradation of cresols

The biodegradation of *p*-cresol in simulated wastewater was carried out using *P. putida* immobilized in PVA gel, in batch and continuous modes in SBBR to evaluate the effects of several operating conditions. The effects of initial substrate concentration, temperature, solution pH and PVA volume fraction on the biodegradation of *p*-cresol were evaluated in a batch SBBR. The same trends for the effects of these conditions were observed as for phenol indicating a high dependence of the biodegradation capabilities of *P. putida* on temperature, pH and biomass abundance, which is represented by PVA volume fraction. The process was optimized at 35°C, 8 and 40%, respectively. As for the effect of the initial substrate concentration, there was no sign of inhibition up to a substrate concentration of 200 mg/l, which justifies the Monod non-inhibitory model (Eq. (7)). Continuous operation is essential when considering the industrial applicability of biodegradation process. Thus, the effects of air flow rate and residence time (as affected by liquid flow rate) were investigated in continuous operation. Continuous biodegradation results indicated that *P. putida* proved to be effective for the biodegradation of *p*-cresol up to 200 mg/l, with a removal efficiency of more than 85% and with the steady state achieved within one residence time. Optimal air flow rate was 2 l/min, with the same observation as for phenol that the biodegradation rate could not be improved beyond a certain air flow rate, which could be due to slugging and bubble coalescence. The increase in the liquid flow rate reduced the residence time inside the reactor and consequently the biodegradation rate was reduced. Assuming *p*-cresol is the limiting reactant and perfect mixing in the bioreactor, which is the case in SBBR, the global biodegradation rate was calculated based on the specific consumption rate and the mass balance of the continuous flow reactor is expressed as:

$$\frac{d M_A}{dt} = F(S_{Ao} - S_A) - r_A V \tag{9}$$

where M_A is the mass (mg) of substrate A in the reactor, t is the time (h), F is the volumetric flow rate (l/h), S_A is the substrate concentration at a certain time, $-r_A$ is the rate of removal of substrate A (mg/l h) and V is the reactor volume (l). Dividing the equation by V:

$$\frac{dS}{dt} = \frac{F}{V}(S_o - S) - r_s \tag{10}$$

The substrate uptake is given by:

$$-r_s = q \tag{11}$$

Assuming constant yield Y is constant, which is valid if the substrate concentration is much higher than K_s (i.e. $S \gg K_s$) [26], the Monod model can then be expressed in terms of the degradation rate q (mg/l h) as:

$$q = \frac{q_{max} S}{K_s + S} \tag{12}$$

Combining Eqs. (10) and (12), the change of substrate concentration in the reactor can be evaluated as:

$$\frac{dS}{dt} = \frac{F}{V}(S_o - S) - \frac{q_{max} S}{K_s + S} \tag{13}$$

The experimental results from the continuous biodegradation process showed very good fit with the model proposed in Eq. (13). The effects of temperature, pH and initial substrate concentration were also evaluated for the other two isomers, o- and m-cresol. The optimum ranges were 30–35°C for temperature and 6–8 for pH, with different optimum values for each isomer. The immobilized *P. putida* could sustain cresols concentration up to 200 mg/l, but substrate inhibition was observed in the biodegradation of o-cresol at 150 mg/l, which could be attributed to the formation of toxic intermediates.

A RWW treatment plant generally receives influents with mixtures of recalcitrant organic chemicals. This is a complex situation where biodegradation of a compound could be strongly affected by the presence of other components in the mixture. This is particularly related the biological transformation called co-metabolism, which involves the transformation of a non-growth substrate by cells growing on a growth substrate [35]. It could also be affected by the accumulation of metabolic intermediates. For a treatment scheme to be effective, all the interactions among the different substrates need to be considered. Scant information is available in the literature on the biodegradation of mixtures of cresols. The potential of *P. putida* in degrading binary (o- and p-cresol) and ternary (o-, p- and m-cresol) mixtures of cresols was also investigated in the continuous mode. It was obviously shown that the biodegradation of o-cresol was strongly inhibited by the presence of p-cresol and m-cresol.

Reference is made to the studies by Surkatti and El-Naas [45, 52] for details on the biodegradation of cresols.

5.3.3. Biodegradation of chlorophenols

The work presented in this section aimed at developing an integrated system for biodegradation of chlorophenols, considering 2,4-dichlorophenol (2,4-DCP) as a model contaminant. The system utilizes the advanced technique for the immobilization of *P. putida* in PVA gel matrix, in a specially designed SBBR that promises to be effective in industrial applications for the treatment of real RWW. Details on this work can be found elsewhere [53, 54].

Batch experiments were performed to confirm that the removal efficiency was due to the biodegradation effect and not to other abiotic effects such as stripping or adsorption on the carrier. It was confirmed that the contribution by these factors to the reduction in DCP concentration ranged from 2.5 to 7%, which confirms the biodegradation effect by immobilized *P. putida.*

Process optimization is critical for any large-scale industrial application. A preliminary screening led to approaching the optimum range for each of the main factors, namely temperature, pH and initial substrate concentration. The full potential of response surface methodology (RSM) with Box-Behnken design was consequently utilized to determine the effects of significant parameters, and their interactions, in the removal of 2,4-DCP and to specify the optimal conditions for its degradation based on the degradation rates achieved in the SBBR batch experiments. A quadratic regression model that is capable of predicting the rate in terms of the main independent variables was developed. The maximum biodegradation rate within the experimentation region was determined as: temperature 32.6°C, pH 5.0 and the initial DCP concentration was 70.5 mg/l. Under these conditions, the predicted global biodegradation rate was 41.8 mg/l h. By analysis of variance, the model showed a good fit with R^2 and adjusted R^2 94.03 and 83.28%, respectively, which justifies the adequacy of the model. It was further experimentally validated. In the batch process, 100% removal of 2,4-DCP was achieved for all DCP initial concentrations, up to 200 mg/l. This is a compound that is naturally difficult for biodegradation, and for which removal efficiencies in the range of 40–60% have often been reported. *P. putida* showed good adaptation to the contaminant with repeated use and showed continued improvement; its degradation capability almost doubled after 4 months of repeated use from 38 to 70 mg/l h. For the sake of comparison, the degradation rates from other reported batch studies are shown in **Table 3**.

Although the biodegradation of 2,4-DCP followed a Haldane inhibition model, the inhibition was not significantly observed at any initial DCP concentration, which could be mainly due to the advantage of the immobilization of bacteria in the PVA gel pellets.

The process was thereafter operated in the continuous mode to investigate the hydrodynamics and the performance of the reactor. The various issues investigated were the hydraulic residence time (*HRT*), the initial concentration of DCP in the feed and the degradation capacity (*DC*), which is a characteristic design factor for continuous reactors. It has an economic significance as a measure of the hydraulic throughput and is defined as:

$$DC = (S_i - S_e)D \tag{14}$$

Initial DCP concentration (mg/l)	Degradation rate (mg/l h)	% Removal	Ref.
1000 (fungi), free	8.3	100	[55]
500 (fungi), free	5.21	100	[56]
113	28.75	100	[57]
120, free	0.48	40	[22]
80, free	2.67	100	[31]
75	70	100	[54]

Table 3. Degradation rates of 2,4-DCP from batch studies in the literature [54].

where S_i and S_e are influent and effluent substrate concentrations, respectively, and D is the dilution rate ($1/HRT$).

Another criterion often used in continuous operations is the organic loading rate (OLR) of the feed that is related to the amount of contaminant that can be processed over a given period of time, defined as:

$$OLR = \frac{S_i F}{V} = \frac{S_i}{HRT} = S_i D \tag{15}$$

ORL is directly affected by changes in the influent flow rate and its initial concentration.

OLR and DC values from the combined sets of experiments (those for the effect liquid flow rate and those for the effect of initial DCP concentration) were plotted in **Figure 1**, with
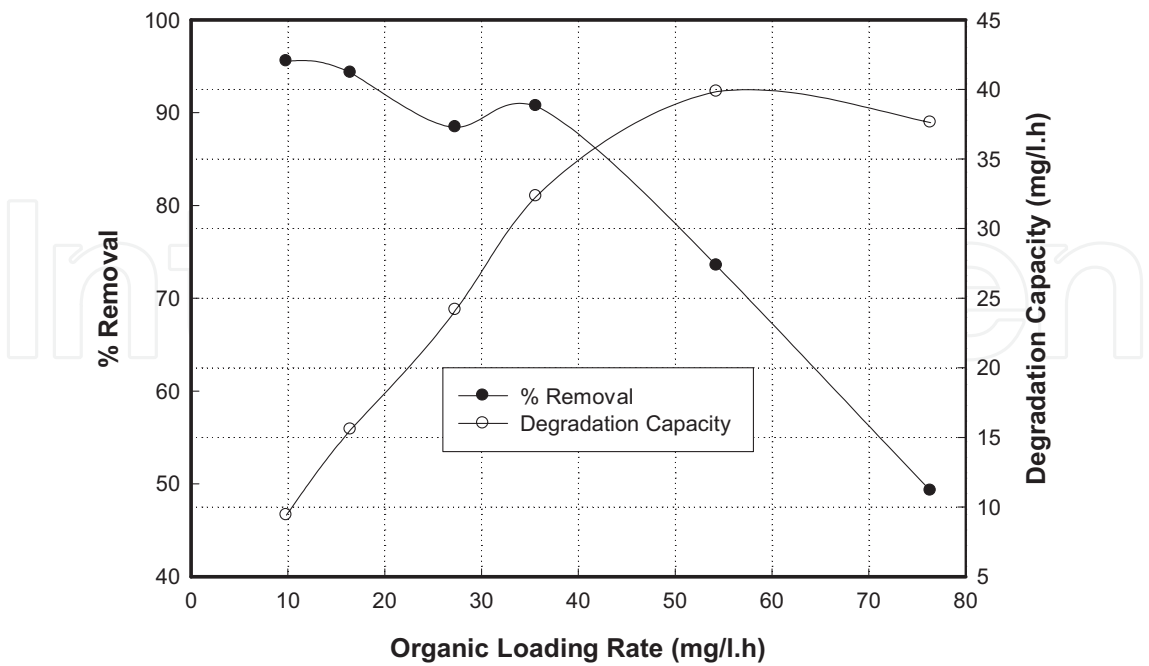

Figure 1. Effect of organic loading rate on degradation capacity and percent removal [54].

corresponding percent removal efficiencies. The results demonstrate that operating conditions are selected with a compromise based on either the desired degradation efficiency (percent removal) or the desired degradation capacity. This is different for a batch operation, in which a bioreactor is operated with a predetermined percent of substrate removal of substrate percent. A combined 80% removal at a throughput of 1400 g/m^3 day was obtained during continuous operation with a *HRT* of 1 h and an initial DCP concentration of 75 mg/l.

Dynamic modeling of DCP degradation in continuous operation was based on the same discussion that applied for cresols and led to the derivation of Eq. (13). However, the Monod expression was replaced with the Haldane one in the equation to give Eq. (16):

$$\frac{\mathrm{d}S}{\mathrm{d}t} = \frac{F}{V}(S_o - S) - \frac{q_{max}\, S}{K_s + S + \left(\frac{S^2}{K_i}\right)} \tag{16}$$

The experimental results from the continuous biodegradation process showed very good fit with the model proposed in Eq. (16). The performance of the SBBR was further tested in the continuous operation for tolerance to organic and hydraulic shock loads, resulting from concentration and liquid flow rate fluctuations. The SBBR showed stability and sustainability, as it could withstand a 100% increase in organic loading rate and recover quickly to its normal operation once the shock load had been terminated. The SBBR performance was also compared to that of a packed bed reactor and proved to be more stable and achieved higher removal efficiencies (77% versus 53%) with a HRT of 1 h and an initial DCP concentration of 75 mg/l. Such a difference between the two reactors is expected as explained by the better mixing properties of the SBBR and the mass and heat transfer limitations of the packed bed reactor.

The process was tested for the treatment of real RWW with characteristics similar to those given in **Table 2**. The immobilized bacteria were acclimatized to the RWW and then used for the batch treatment in the SBBR. The process was shown to be effective, with 100% removal efficiencies achieved for all cresols, whereas the removal efficiencies for phenol and DCP were approximately 87% and 63%, respectively. Significant overall reduction in total phenols of almost 90% was observed and a maximum COD reduction of 59%. These results are merely due to the biodegradation effect without any pre- or post-treatment. With this in mind, they may be considered quite satisfactory.

6. Conclusions

The presence of organic contaminants in RWW represents a major environmental concern for the oil industry. The current options for RWW management are rather economically or technically limited in application. A new approach that utilizes a state of the art technique for the immobilization of an effective organics-degrading bacterial strain (*P. putida*) in a specially designed spouted bed bioreactor has proven to be effective for the treatment of RWW.

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