

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# Denitrification in the Presence of Chlorophenols: Progress and Prospects

---

Emir Martínez-Gutiérrez, Anne-Claire Texier,  
Flor de María Cuervo-López and Jorge Gómez

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.68860>

---

## Abstract

Diverse industrial effluents may contain recalcitrant compounds such as chlorophenols. Besides, excessive use of pesticides in agriculture is a major cause of the appearance of chlorophenols in surface and groundwater. To mitigate and control the effects of chlorophenols in the environment, various methods have been developed for their elimination. Biological processes represent a sustainable and economical alternative that can lead to the mineralization of chlorophenols and be effective for the removal of these pollutants from different water bodies, such as rivers, groundwater, and wastewater. Some studies have reported that chlorophenols mineralization and nitrate reduction may simultaneously be performed. Other works have suggested that a reductive dechlorination occurs such as the first step and later, the phenol formed is subsequently mineralized by denitrification. However, the published information can be confusing as the denitrifying process is often associated with the sole nitrate consumption without corroborating the total reduction of nitrate to  $N_2$ . Additionally, there are alternative systems that combine biological process with a chemical or electrochemical process for chlorophenols removal. This chapter focuses on the advances accomplished in the study of the removal of chlorophenols under denitrifying conditions with the aim of having a clearer panorama of the treatment alternatives that can be applied for treatment of this type of effluents.

**Keywords:** denitrification, chlorophenols, rates, anaerobic, combined systems

---

## 1. Introduction

Human activities generate effluents from production processes and domestic activities which may contain nitrogen and carbon pollutants. This pollution alters the global nitrogen and carbon cycles. Inorganic nitrogen is mainly present in the aqueous effluents such as nitrate, nitrite, and ammonium, causing serious problems to ecosystems and to public health.

These compounds can achieve high levels of toxicity to aquatic organisms and may promote the growth of aquatic plants, which accelerate the eutrophication process of water bodies [1]. The ingestion of nitrite and nitrate by infants can promote methemoglobinemia and the formation of nitrosamines, which might be carcinogens [2]. On the other hand, diverse industrial effluents may contain recalcitrant compounds such as chlorophenols, which are derivatives of phenol that contain one or more covalently bonded chlorine atoms. Chlorophenols have been utilized for wood preservation, as well as for manufacturing of pesticides, antiseptics, and dyes. However, the excessive use of pesticides in agriculture is one of the major causes of the appearance of chlorophenols in surface and groundwater [3]. Depending on their concentration, they can be toxic compounds, causing damage to the cell membranes as well as uncoupling oxidative phosphorylation [4].

To diminish the adverse effects of chlorophenols in the environment, various methods have been developed for their elimination, including physical, chemical, electrochemical, and biological processes. The first three methods appear to be faster, but everything indicates that they are expensive and generate collateral contamination, making them less environmentally friendly processes than the biological treatment. Biological processes represent a sustainable and cost-effective alternative that can lead to the mineralization of chlorophenols and can be effective for the removal of these pollutants from different water bodies, such as rivers, groundwater, and wastewater. Most of the information on disposal of chlorophenols under anaerobic conditions has been obtained under methanogenic conditions. There is evidence that shows that removal of chlorophenols by methanogenic microbial consortia is initiated by a reductive dechlorination and ends with the formation of methane and  $\text{CO}_2$  [5], although more chlorinated chlorophenols are not always completely mineralized and less chlorinated compounds are obtained as end products [6]. Chlorophenol mineralization coupled to denitrification is still poorly documented. In this regard, there are few studies showing the possibility of chlorophenol consumption coupled to reduction of nitrate, although it is suggested that the pathway is different to reductive dechlorination [7]. Other studies suggested that reductive dechlorination occurs at first and later the phenol formed is subsequently mineralized by denitrification process [8]. However, the published information can be confusing, as the denitrifying process is often associated with the sole nitrate consumption without corroborating the total reduction of nitrate to  $\text{N}_2$ .

Considering that efficient removal of recalcitrant compounds such as chlorophenols requires detailed analysis, this chapter focuses on the advances accomplished in the study of the removal of chlorophenols under denitrifying conditions. First, the physicochemical characteristics of the chlorophenols that confer their recalcitrant and toxic properties are presented. Then, general aspects of denitrification, such as microbiology and biochemistry, as well as the influence of various environmental factors, are presented. In physiological terms, the elimination of chlorophenols under denitrifying conditions is discussed, presenting the different configurations of reactors studied, types of inoculum, as well as the different strategies used to increase their consumption. Finally, the recently studied systems that combine the biological process with a chemical or electrochemical process, in order to increase the consumption of chlorophenols without the generation of toxic waste, are also presented.

## 2. Physicochemical properties of chlorophenols

Chlorophenols are organochlorine compounds whose structure consists of a phenol and one or more chlorine atoms that are covalently bonded. In total, there are 19 types of chlorophenols differing from each other in the amount and position of the chlorine atoms. They can be subdivided into five groups: monochlorophenols, dichlorophenols, trichlorophenols, tetrachlorophenols, and pentachlorophenols. Most chlorophenols are solid at room temperature, with the exception of 2-chlorophenol (2-CP) which is liquid. They are compounds with strong odor and medicinal taste with very low organoleptic thresholds, being perceived in water at very small quantities ( $\mu\text{g/L}$ ). Chlorophenols present high log Kow (octanol water partition coefficient) values and low solubility in water (**Table 1**). As chlorination level increases, their water solubility decreases and their acidity increases. Similarly, the log Kow also increases with the number of chlorine atoms, favoring their bioaccumulation [9]. Transport and transformation of chlorophenols in natural environments depend on pH, oxygen concentration, presence of other organic and inorganic substances, and temperature as well as their own structure [10].

Apparently, toxicity of chlorophenols is related to the degree of chlorination and the proximity of chlorine to the hydroxyl group. Chlorophenols with chlorine in the *ortho* position show lower toxicity than chlorophenols with the same number of chlorine in the *meta* or *para* position [11]. Toxicity of chlorophenols may also be related to their log Kow [12], as toxicity increases with a higher lipophilicity. Toxic effects of chlorophenols have been related to membrane destruction and inhibition of oxidative phosphorylation. This blockade of oxidative phosphorylation can occur by different ways: interfering with the release of hydrogen to the electron transport chain, inhibition of the transfer of electrons along the electron transport chain to oxygen, interference with the release of oxygen to the terminal electron carrier, or inhibition of the activity of adenosine triphosphate (ATP) synthase [11].

	2-CP	4-CP	2,4-DCP	2,4,6-TCP	2,3,5,6-TTCP	PCP
Molecular weight (g/mol)	128.56	128.56	163.0	197.45	197.45	266.34
Solubility at 20°C (g/L)	28	27	4.5	0.434	0.434	0.014
Density (g/cm <sup>3</sup> )	1.262	1.2238	1.38	1.49	1.84	1.98
Log Kow	2.29	2.4	3.17	3.7	4.9	5.02
Vapor pressure at 20°C (mm Hg)	0.99	0.23	0.14	0.03	0.0059	0.0002
Melting point (°C)	9.4	42–44	42–43	69	115	191
Boiling point (°C)	174.9	217	210	246	288	309
pKa	8.56	9.18	7.68	6.0	5.5	5.01

CP: chlorophenol, DCP: dichlorophenol, TCP: trichlorophenol, TTCP: tetrachlorophenol, and PCP: pentachlorophenol.

**Table 1.** Physical and chemical properties of chlorophenols.

### 3. Microbiology of denitrification

In order to carry out denitrification, which is defined as the biological dissimilative transformation of nitrate ( $\text{NO}_3^-$ ) or nitrite ( $\text{NO}_2^-$ ) into molecular nitrogen ( $\text{N}_2$ ) under anoxic conditions with energy conservation [13], an electron donor is required. Therefore, denitrifying microorganisms must have the ability for using nitrate or nitrite as electron acceptors to reduce them to molecular nitrogen. Organotrophic or autotrophic microorganisms are involved in denitrification process depending on their ability to use organic or inorganic compounds as electron sources, respectively. Their remarkable characteristic is their facultative anoxic respiration.

Distribution of denitrifying microorganisms in nature is ubiquitous. Organotrophic and autotrophic denitrifiers belong to  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\epsilon$ -proteobacteria group which comprise both, Gram-negative and Gram-positive bacteria. Nevertheless, some members of Archae and Eukarya have shown the ability for reducing nitrate to  $\text{N}_2$  [14, 15]. Most of organotrophic and autotrophic denitrifiers grow under neutral and mesophilic conditions [16, 17]. Organotrophic denitrifiers have been found in natural ecosystems as soil [18], surface water [19], groundwater, and sediments [20]; in wastewater treatment plants; and in different types of reactor configurations treating synthetic wastewater under organotrophic [21], autotrophic [22], or mixotrophic conditions, where mixtures of both organic and inorganic electron sources are present [22, 23]. For illustration purposes, several denitrifying microorganisms and their physiological characteristics are included in **Table 2**.

Group	Genus/species	Electron donor	Physiological characteristics	Reference
$\alpha$ -Proteobacteria	<i>Paracoccus/P. pantotrophus</i>	Organic and sulfur compounds, $\text{H}_2$	Organotrophic, sulfur and hydrogen autotrophic denitrification	[16, 24]
$\beta$ -Proteobacteria	<i>Thiobacillus thiophilus</i>	Sulfide, sulfur	Sulfur autotrophic denitrification	[25]
	<i>Azoarcus</i>	Organic compounds	Organotrophic denitrification	[23, 26, 27]
	<i>Thauera</i>	Acetate, sulfide, $\text{H}_2$	Organotrophic, sulfur and hydrogen autotrophic denitrification	[21, 23]
	<i>Acidovorax spp.</i>	Glucose, acetate, $\text{H}_2$	Organotrophic and hydrogen autotrophic denitrification	[21, 28, 29]
	<i>Flavobacterium spp.</i>	Glucose, acetate	Organotrophic denitrification	[21, 28]
$\gamma$ -Proteobacteria	<i>Pseudomonas sp.</i>	Organic compounds, $\text{H}_2$	Organotrophic and hydrogen autotrophic denitrification	[24]
	<i>Acinetobacter sp.</i>	$\text{H}_2$	Hydrogen autotrophic denitrification	[30]
	<i>Aeromonas sp.</i>	$\text{H}_2$	Hydrogen autotrophic denitrification	[31]
$\epsilon$ -Proteobacteria	<i>Sulfurimonas lithotrophicum</i>	Sulfur	Sulfur autotrophic denitrification	[32]
	<i>Thiomicrospira CVO</i>	Sulfur, $\text{H}_2$	Sulfur and hydrogen autotrophic denitrification	[33]

**Table 2.** Some denitrifying microorganisms and their physiological characteristics.

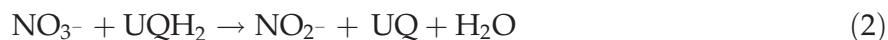
Genus *β-proteobacteria* has been found dominant in many denitrification systems [34]. *Thauera* is a dominant Gram-negative organotrophic bacterium belonging to *β-proteobacteria* which has been identified in wastewater treatment systems [35], in an integrated system of three-dimensional biofilm-electrode reactor and sulfur autotrophic denitrification (3DBER-SAD) under mixotrophic conditions [27]. *Thauera* has also been identified in sequential batch reactors where the heterotrophic and autotrophic denitrifying process was conducted [23] and in several denitrifying bioreactors under autotrophic conditions, suggesting its ability for autotrophic growth [23]. *Acidovorax* is a Gram-negative bacterium which has the ability of using both acetate and hydrogen for denitrification [29]. Denitrifying bacteria, similar to *Acidovorax* and *Azoarcus*, a facultatively anaerobic, mesophilic, and Gram-negative bacterium with the ability of growing with a variety of organic substrates [26], have been identified under mixotrophic denitrifying conditions [27]. Denitrifying species of *Acidovorax* spp. and *Flavobacterium* spp. have been detected in a soil column system amended with glucose [21]. Recently, the ability of *Pseudomonas* sp. C27 for conducting both organotrophic and autotrophic denitrification has been reported [22]. On the other hand, *Thiobacillus denitrificans*, an obligate autotrophy and facultative anaerobic bacterium, which can use elemental sulfur as an electron donor, has been isolated from natural environments, man-made environments [17], and denitrifying reactors operated under mixotrophic conditions [27].

#### 4. Biochemical aspects

Irrespective of whether the organic or autotrophic process is conducted, the denitrification process has been described as a modular organization in which every biochemical reaction is catalyzed by a specific reductase [36]. These reactions occur when no oxygen is available and the environment becomes anoxic [37]. According to Mariotti [38], the denitrification process can be described as Eq. (1) indicates.



This general equation can be decomposed into four enzymatic reactions. At first, nitrate is reduced to nitrite by nitrate reductase (*Nar*) (Eq. (2)). The reaction can take place in the cell membrane and periplasmic space. Affinity constant (*Km*) ranging from 0.15–15 mM and  $\Delta G^{\circ'}$  of  $-163.2 \text{ KJ/reaction}$  values have been reported for this reaction [39, 40]. UQH<sub>2</sub> corresponds to reduced ubiquinone, UQ to ubiquinone, c<sup>2+</sup> to reduced cytochrome, and c<sup>3+</sup> to oxidized cytochrome.

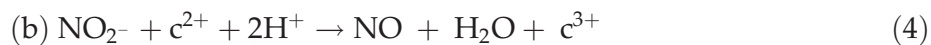


A subsequent reduction of nitrite to nitric oxide is carried out by one of two nitrite reductases (*Nir*, *CuNir*) or the cytochrome *cd<sub>1</sub>*, both located at the periplasmic space (Eq. (3) and (4)). *Km* values of 3.13–750 μM [41, 42] and 6–46 μM [39, 41] are reported for *Nir*/*CuNir* or *cd<sub>1</sub>*, respectively, whereas  $\Delta G^{\circ'}$  of  $-73.2 \text{ KJ/reaction}$  correspond to this stage.





or



Afterward, in the cell membrane, nitric oxide is reduced to nitrous oxide by the enzyme nitric oxide reductase (*Nor*) (Eq. (5)). *K<sub>m</sub>* values of 0.25–60  $\mu\text{M}$  are reported for *Nor* enzyme with a  $\Delta G^{\circ'}$  of  $-306.3 \text{ KJ/reaction}$  [43, 44].



Finally, nitrous oxide is reduced to  $\text{N}_2$  by the enzyme nitrous oxide reductase (*Nos*), which is located at the periplasmic space (Eq. (6)). *K<sub>m</sub>* values of 2–6  $\mu\text{M}$  are reported for this enzyme with a  $\Delta G^{\circ'}$  of  $-306.3 \text{ KJ/reaction}$  [45].



## 5. Denitrification and its environment

Denitrification performance is controlled by many environmental factors such as concentration, type and solubility of the substrate, C/N ratio, temperature, and pH, among other factors. These environmental variables determine the metabolic behavior, being the effect of each factor different on the biochemistry and physiology of the microorganisms [39, 46]. In this regard, experimental data have suggested that a C/N ratio close to the stoichiometric value is required for complete denitrification [47]. In this respect, many authors have made recommendations to adjust the C/N, S/N ratio for denitrification processes [36, 48]. Tiedje [49] observed that an excess of reducer source induced the reduction of nitrate to ammonium. Denitrification is an exergonic process where the energy formation depends on the type of reducer source. Degradation of monochlorophenols coupled to denitrification is also an exergonic process (Table 3). This makes denitrification a potential microbial biomass producer. Nonetheless, wastewater treatment should be a dissimilatory process where the pollutants might be essentially removed through catabolic processes.

Oxygen is generally considered as a denitrifying inhibitor [50]. Likewise, according to  $\text{O}_2$  and nitrate potential redox, a competition effect can occur between these oxidants. It has been reported that nitrate could be reduced even in the presence of  $\text{O}_2$  [51]. On the other hand, the denitrifying process can be carried out in a temperature range between 5 and  $35^\circ\text{C}$ . However, it has been observed that at low temperatures, the emissions of nitrous oxide increase whereas  $\text{N}_2$  formation decreases [52].

pH is an independent variable that affects denitrification process at different levels [46, 53]. The common pH value employed for denitrification is around 7. At low pH values, an inhibition on the reduction of nitrous oxide occurs, causing an accumulation of nitrous oxide and a decrease

Compound	Equation	$\Delta G^{\circ}$ (KJ/reaction)
Acetic acid	$\text{CH}_3\text{COOH} + 1.6\text{NO}_3^- \rightarrow 2\text{CO}_2 + 0.8\text{N}_2 + 1.6\text{OH}^- + 1.2\text{H}_2\text{O}$	-843
Glucose	$\text{C}_6\text{H}_{12}\text{O}_6 + 4.8\text{NO}_3^- \rightarrow 2.4\text{N}_2 + 6\text{HCO}_3^- + 1.2\text{H}^+ + 2.4\text{H}_2\text{O}$	-2686
Phenol	$\text{C}_6\text{H}_6\text{O} + 5.6\text{NO}_3^- + 0.2\text{H}_2\text{O} \rightarrow 2.8\text{N}_2 + 6\text{HCO}_3^- + 0.4\text{H}^+$	-2818
Methanol	$\text{CH}_3\text{OH} + \text{NO}_3^- \rightarrow 0.5\text{N}_2 + \text{CO}_2 + 2\text{H}_2\text{O}$	-582
<i>p</i> -Cresol	$\text{C}_7\text{H}_8\text{O} + 6.8\text{NO}_3^- \rightarrow 3.4\text{N}_2 + 7\text{HCO}_3^- + 0.2\text{H}^+ + 0.4\text{H}_2\text{O}$	-3422
Toluene	$\text{C}_7\text{H}_8 + 7.2\text{NO}_3^- + 0.2\text{H}^+ \rightarrow 3.6\text{N}_2 + 7\text{HCO}_3^- + 0.6\text{H}_2\text{O}$	-3524
Xylene	$\text{C}_8\text{H}_{10} + 8.4\text{NO}_3^- + 0.4\text{H}^+ \rightarrow 4.2\text{N}_2 + 8\text{HCO}_3^- + 1.2\text{H}_2\text{O}$	-4136
Sulfide	$\text{S}^{2-} + 2\text{NO}_3^- + 4\text{H}^+ \rightarrow \text{SO}_4^{2-} + \text{N}_2 + 2\text{H}_2\text{O}$	-922
Monochlorophenol	$\text{C}_6\text{H}_5\text{ClO} + 5.2\text{NO}_3^- + 1.4\text{H}_2\text{O} \rightarrow 2.6\text{N}_2 + 6\text{HCO}_3^- + 1.8\text{H}^+ + \text{Cl}^-$	-2742

**Table 3.** Stoichiometric reactions of the denitrifying respiratory process using different electron sources and their  $\Delta G^{\circ}$  values (according to Cuervo-López et al. [36]).

in  $\text{N}_2$  formation [54, 55]. Denitrification can also be influenced by the speciation and bioavailability of the chemical compounds used as reducer sources. Thus, physicochemical conditions must be controlled in order to have a faster and efficient denitrifying process.

## 6. Biodegradation of chlorophenols under denitrifying conditions

Chlorophenols are generally degraded under anaerobic conditions through the first reductive dechlorination step, which consists of the substitution of chlorine atoms by hydrogen atoms (Eq. (7)).



This stage is catalyzed by specific dehalogenases enzymes. The majority of the known reductive dehalogenases belong to the CprA/PceA family and contain one corrinoid and two iron-sulfur clusters as cofactors [56]. Reductive dechlorination requires the addition of electron donors. There are other cases in which chlorophenols are used as carbon and energy sources for microorganisms [57]. Under methanogenic conditions, mineralization of various chlorophenols to  $\text{CO}_2$  and methane has been observed [5]. However, it is unclear if reductive dechlorination would be involved in the degradation of chlorophenols under denitrifying conditions. In fact, different pathways that do not involve the dechlorination reductive step have been suggested [7].

The study of chlorophenols under denitrifying conditions has been mainly evaluated using monochlorophenols. Chang et al. [58] used a biofilm to remove 2-CP under denitrifying conditions in batch cultures. They observed that the nitrate disappeared in 16 h, and there was a consumption of 2-CP. However, there was no formation of phenol in this period, suggesting that 2-CP was not dechlorinated in the presence of nitrate. Phenol was produced only after the disappearance of nitrate, suggesting that nitrate competed with 2-CP as an



electron acceptor. A similar behavior was observed by Sanford and Tiedje [8], who evaluated, in serological bottles, the elimination of 2-CP in the presence of nitrate and acetate. They observed that the consumption of 2-CP was inhibited by the presence of nitrate and was only carried out when nitrate disappeared or was found in concentrations lower than 104 mg/L. Yu et al. [59] studied the effect of nitrate addition on the reductive dechlorination of pentachlorophenol (PCP) and found that low concentrations of nitrate (0–62 mg/L) can enhance reductive dechlorination of PCP, whereas high concentrations (310–1860 mg/L) provoke a contrary effect. Thus, reductive dechlorination could be carried out at low concentrations of nitrate. On the other hand, Häggblom et al. [60] studied the removal of three monochlorophenols in batch cultures under denitrifying conditions. Only 2-CP was eliminated in 110 days; nevertheless, they did not detect the formation of phenol as a product of reductive dechlorination. Bae et al. [7] also studied the elimination of monochlorophenols and dichlorophenols under denitrifying conditions in batch cultures, finding that 4-chlorophenol (4-CP), 2,4-dichlorophenol (2,4-DCP), and 2,6-dichlorophenol (2,6-DCP) were not biodegraded, whereas 2-CP and 3-chlorophenol (3-CP) were mineralized and the presence of nitrate was essential. The authors reported that 2-CP was oxidized to  $\text{CO}_2$  under denitrifying conditions and suggested the presence of a population capable of eliminating 2-CP by a mechanism that does not involve reductive dechlorination.

As observed in **Table 4**, most of the studies with chlorophenols have been carried out in batch assays and only few types of reactors have been evaluated under denitrifying conditions. Moussavi et al. [61] evaluated the elimination of 2-CP in a granular anoxic baffled reactor (AnBR) increasing the concentration of 2-CP up to 500 mg/L without affecting the efficiency of 2-CP removal, so this could be a feasible process at low cost. Wang et al. [62] evaluated the removal of PCP in a packed reactor with corncob as both carbon source and biofilm support and obtained efficiencies of nitrate and PCP removal above 90%.

In conclusion, mineralization of chlorophenols coupled to denitrification is rarely documented as the total oxidation of chlorophenols to  $\text{CO}_2$  and reduction of nitrate to  $\text{N}_2$  have not been corroborated. The available information is controversial as several works evidenced that the presence of nitrate inhibits the transformation of chlorophenols [8, 63], while other authors indicate that reductive dechlorination can be carried out at low concentrations of nitrate [59]. In fact, other studies evidenced that mineralization of chlorophenols is linked to denitrification, and the presence of nitrate was necessary for the biodegradation [7, 64]. In addition, the denitrifying process is often evaluated by the sole nitrate consumption without verifying its total reduction to  $\text{N}_2$ . Therefore, it is necessary to carry out more studies which evaluate the process through response variables such as removal efficiencies, yields of product formation, and rates in order to characterize and better understand the process.

### 6.1. Strategies for improving the consumption of chlorophenols

It has been pointed out that the main difficulty for the elimination of chlorophenols is the strong stability that the carbon-halogen bond of the aromatic compound confers to its structure [67]. Thus, in many cases, the biodegradation is slow. Several strategies for increasing the consumption

Chlorophenol (mg/L)	Type of reactor	Inoculum	Electron donor	Removal efficiency of CPs	Removal efficiency of nitrate	Products	Reference
2-CP (12.8)	Batch (serum bottle)	Sediment	Na <sub>2</sub> S.9H <sub>2</sub> O	—	—	—	[60]
2-CP, 3-CP, or 4-CP (25.7)	Batch	Soil	Acetate, volatile fatty acids	—	—	Phenol, benzoate	[8]
2-CP (12.8)	Batch	Acclimated sludge	Na <sub>2</sub> S.9H <sub>2</sub> O	—	—	—	[7]
3-CP (2.0–5.2)	Up-flow columns	Activated sludge	Na <sub>2</sub> S.9H <sub>2</sub> O	27–100%	—	Phenol, benzoate	[65]
2-CP(25)	Batch (gas-permeable silicone membrane bioreactor)	Hydrogenotrophic biofilm (acclimated)	H <sub>2</sub>	Around 100%	Around 100%	Phenol	[58]
4-CP, 2,4 DCP (5)	Sequencing batch reactors	Acclimated biomass	Milk powder plus yeast extract	—	—	—	[66]
PCP (5 mg/L)	Laboratory-scale reactor packed	Biofilm	Corncob	40–91%	98%	3-CP, phenol	[62]
2-CP (50–500)	Anoxic baffled reactor	Activated sludge (enrichment)		>99%	—	—	[61]
PCP (1–5)	Batch (serum bottles)	Soil	Lactic acid	Around 100%	Around 100%	—	[59]

**Table 4.** Biodegradation of different chlorophenols under denitrifying conditions.

of chlorophenols have been proposed, although most of them have been conducted under aerobic and anaerobic conditions and in minor proportions under denitrifying conditions.

Some strategies have been proposed to increase the efficiency and/or rate of chlorophenols consumption. These include the sludge adaptation to pollutants, the use of genetically modified microorganisms, and the addition of alternative carbon sources [68]. It has been also suggested that the addition of readily oxidized carbon sources could exert various beneficial effects, such as decreasing toxicity, acting as an enzyme-inducing agent, or providing reducing power for the consumption of recalcitrant organic compounds [69–71]. Furthermore, Puyol et al. [72] observed accumulation of different intermediates depending on the co-substrate used. When methanol, ethanol, or volatile fatty acids were used as co-substrates, 4-chlorophenol was accumulated while 2,4-dichlorophenol was accumulated when lactate was used as the co-substrate.

Under denitrifying conditions, Hu et al. [66] found that the presence of co-substrates caused a significant decrease in the degradation rate of 4-chlorophenol (by 4 times) while the biodegradation rate of 2,4-dichlorophenol increased by 4.2 times. Therefore, it could be said that the use of co-substrates does not always have a positive effect on the biodegradation of recalcitrant compounds. The compounds used as co-substrates include compounds of easy oxidation and

compounds with a structure similar to chlorophenols. Regarding this, Martínez-Gutiérrez et al. [73] evaluated the effects of phenol and acetate on the mineralization of 2-CP by a denitrifying sludge in batch assays. When phenol was used as a co-substrate, the specific rate of 2-CP consumption increased by 2.6 times, regarding to a control assay without co-substrate. When acetate was used, the specific rate of 2-CP consumption increased by 9 times, suggesting that the addition of co-substrates is a good alternative for improving the biodegradation of chlorophenols. These results also suggest that the effects of co-substrates addition depend on several factors: type of both the co-substrate and chlorophenol employed, inoculum source, and experimental conditions.

## 7. Coupled systems for chlorophenol degradation

Recently, other strategies have been developed for the elimination of recalcitrant compounds using systems that combine advanced oxidation (AOP) or electrochemical processes with biological processes. Daghighi et al. [74] evaluated the degradation of toluene using bio-electrochemical reactors obtaining a current power of 431 mA/m<sup>2</sup>. Yeruva et al. [75] evaluated the integration of a sequencing batch reactor (SBR) and a bio-electrochemical treatment system (BET) for the treatment of petrochemical wastewater under anoxic conditions, obtaining high degradation and power generation (17.12 mW/m<sup>2</sup>). The application of an electrochemical treatment can diminish the time required for the treatment of chlorinated pesticides in the biological process [76]. A sequential biological advanced oxidation process was used for the degradation of 2,4-dichlorophenol, consisting of an up-flow anaerobic sludge blanket (UASB) reactor and a UV/H<sub>2</sub>O<sub>2</sub>/TiO<sub>2</sub> system, obtaining 52.7% of degradation in only 6 h [77]. However, the degradation of chlorophenols with nitrate using combined systems has been scarcely evaluated. In this sense, Arellano-González et al. [78] evaluated an electrochemical-biological combined system where the reductive dechlorination was carried out in an ECCOCEL-type (Pd-Ni/Ti electrode) reactor that achieved 100% transformation of 2-CP into phenol. Then, the phenol formed was mineralized by a biological denitrification process. The total time required for 2-CP conversion into CO<sub>2</sub> was 7.5 h.

## 8. Perspectives

Biodegradation processes of chlorophenols have been studied extensively because they are more economical and friendly environmental processes in comparison with physicochemical, AOP, and electrochemical processes. The information presented in this review shows that denitrification might be an efficient biological process for the treatment of effluents contaminated with nitrogen and chlorophenols. It has been also reported that biological processes may achieve the complete removal of many types of chlorophenols under aerobic and anaerobic conditions, but they do not always lead to mineralization. It is crucial considering that biodegradation processes can generate more toxic and recalcitrant intermediates

than the original pollutant, and the partial oxidation of recalcitrant molecules should be prevented, favoring their mineralization. In this review, it is shown that recent experimental evidences demonstrated the possibility to use denitrification for 2-CP mineralization associated with the reduction of nitrate to nitrogen gas. These results suggest that denitrification might be used for the mineralization of chlorophenols producing CO<sub>2</sub> and N<sub>2</sub> as final products and obtaining high removal efficiencies. However, more studies on chlorophenols biodegradation by denitrifying processes are needed, especially with mixtures of chlorophenols. More studies on physiological, kinetic, and biochemical aspects of denitrification are also required to identify the limiting steps of the biodegradation metabolic pathways and to better understand how controlling denitrifying processes in bioreactors without the formation of undesirable by-products.

Another important aspect is that it has been shown that chlorophenols biodegradation by denitrifying microorganisms is very slow. As a consequence, the application of denitrification processes for chlorophenols removal is still limited, requiring very long acclimation and retention times, especially for the treatment of wastewaters contaminated with high chlorophenol concentrations. Different treatment alternatives have been proposed in order to increase the rate and efficiency of chlorophenol consumption and among them are adaptation to the pollutants, utilization of genetically modified microorganisms, and addition of alternative sources of energy. However, in spite of the addition of co-substrates, the time required for complete mineralization of chlorophenols can be still very long compared to those obtained in physicochemical processes. In recent years, there have been proposals for coupling oxidation processes (AOP or electrochemical) to biological processes such as denitrification to combine benefits of both types of treatment and establish more efficient, more rapid, less expensive, and environmentally friendly treatment trains for degradation of recalcitrant compounds in wastewater. One alternative is the pretreatment of chlorophenols containing effluents through chemical or electrochemical processes to make them more easily degradable in a sequencing denitrifying biological treatment. Recent results showed that times can be considerably reduced for the complete mineralization of 2-CP in an electrochemical-biological combined system, where an electrocatalytic dehydrogenation process (reductive dechlorination) was coupled to a biological denitrification process in sequential ECCOCEL-type (Pd-Ni/Ti electrode) and rotating cylinder denitrifying reactors. The total time required for 2-CP mineralization in the combined electrochemical-biological process was close to the previously reported times for electrochemical and AOP processes, but in this case, an efficient process was obtained without accumulation of by-products or generation of excessive energy costs due to the selective electrochemical pretreatment. This study showed that the use of electrochemical reductive pretreatment combined with denitrification could be a promising technology for the removal of recalcitrant molecules, such as chlorophenols, from wastewater by more efficient, rapid, and environmentally friendly processes. However, more studies are required in order to get an insight about the denitrification of electrochemically pretreated effluents in different combined systems, different configurations of reactors, and in the presence of different mixtures of chlorophenols and types of co-substrates.

## Author details

Emir Martínez-Gutiérrez<sup>1\*</sup>, Anne-Claire Texier<sup>2</sup>, Flor de María Cuervo-López<sup>2</sup> and Jorge Gómez<sup>2</sup>

\*Address all correspondence to: emir.martinez@inecol.mx

1 CONACYT-Institute of Ecology, Veracruz, México

2 Biotechnology Department, Autonomous Metropolitan University-Iztapalapa, Mexico City, México

## References

- [1] Laws EA. Aquatic Pollution—An Introductory Test. USA: Interscience; 1993. p. 55
- [2] Forman D. Nitrate exposure and human cancer. In: Bogardi I, Kuzelka R, editors. Nitrate Contamination, RD NATO ASI Series. G30 Berlin, Germany: Springer-Verlag; 1991. pp. 281–288. DOI: 10.1007/978-3-642-76040-2\_20
- [3] Olaniran AO, Igbinosa EO. Chlorophenols and other related derivatives of environmental concern: Properties, distribution and microbial degradation processes. *Chemosphere*. 2011;**83**:1297–1306. DOI: 10.1016/j.chemosphere.2011.04.009
- [4] Bradberry SM, Proudfoot AL, Vale JA. Poisoning due to chlorophenoxy herbicides. *Toxicological Reviews*. 2004;**23**:65–73. DOI: 10.2165/00139709-200423020-00001
- [5] Boyd S, Shelton D. Anaerobic biodegradation of chlorophenols in fresh and acclimated sludge. *Applied and Environmental Microbiology*. 1984;**47**:272–277. DOI: 0099-2240/84/020272-06\$02.00/0
- [6] Garibay-Orijel C, Ríos-Leal E, García-Mena J, Poggui-Varaldo M. 2,4,6-Trichlorophenol and phenol removal in methanogenic and partially-aerated methanogenic conditions in fluidized bed reactors. *Journal of Chemical Technology and Biotechnology*. 2005;**80** (10):1180–1187. DOI: 10.1002/jctb.1313
- [7] Bae H, Yamagishi T, Suwa Y. Evidence for degradation of 2-chlorophenol by enrichment cultures under denitrifying conditions. *Microbiology*. 2002;**148**:221–227. DOI: 10.1099/00221287-148-1-221
- [8] Sanford R, Tiedje J. Chlorophenol dechlorination and subsequent degradation in denitrifying microcosms fed low concentration of nitrate. *Biodegradation*. 1996;**7**:425–434. DOI: 10.1007/BF00056426
- [9] Chiou CT, Schmedding DW, Manes M. Improved prediction of octanol-water partition coefficients from liquid-solute water solubilities and molar volumes. *Environmental Science and Technology*. 2005;**39**(22):8840–8846. DOI:10.1021/es050729d



- [10] Czaplicka M. Sources and transformations of chlorophenols in natural environment. *Science of the Total Environment*. 2004;**322**:21–39. DOI: 10.1016/j.scitotenv.2003.09.015
- [11] Pepelko WE, Gaylor DW, Mukerjee D. Comparative toxic potency ranking of chlorophenols. *Toxicology and Industrial Health*. 2005;**21**:93–111. DOI: 10.1191/0748233705th204oa
- [12] Smith S, Furay VJ, Layiwola PJ, Menezes-Filho JA. Evaluation of the toxicity and quantitative structure/activity relationships (QSAR) of chlorophenols to the copepodid stage of a marine copepod (*Tisbe battagliai*) and two species of benthic flatfish, the flounder (*Platichthys flesus*) and sole (*Solea solea*). *Chemosphere*. 1994;**28**(4):825–836. DOI: 10.1016/0045-6535(94)90234-8
- [13] Zumft WG. Cell biology and molecular basis of denitrification. *Microbiology and Molecular Biology Reviews*. 1997;**61**:533–616
- [14] Shoun H, Kim DH, Uchiyama H, Sugiyama J. Denitrification by fungi. *FEMS Microbiology Letters*. 1992;**94**:277–281. DOI: 10.1111/j.1574-6968.1992.tb05331.x
- [15] Hayatsu M, Tago K, Saito M. Various players in the nitrogen cycle: Diversity and functions of microorganisms involved in nitrification and denitrification. *Soil Science and Plant Nutrition*. 2008;**54**:33–45. DOI: 10.1111/j.1747-0765.2007.00195.x
- [16] Robertson LA, Kuenen. *Thiosphera panthotropha* gen. nov. sp. nov., a facultatively anaerobic, facultatively autotrophic sulfur bacterium. *Microbiology*. 1983;**129**:2847–2855. DOI:10.1099/00221287-129-9-2847
- [17] Shao M, Zhang T, Fang HH. Sulfur-driven autotrophic denitrification: diversity, biochemistry, and engineering applications. *Applied Environmental and Biotechnology*. 2010;**88**:1027–1042. DOI: 10.1007/s00253-010-2847-1
- [18] Morris JT, Whiting GJ, Chapelle FH. Potential denitrification rates in deep sediments from the southeastern coastal plain. *Environmental Science and Technology*. 1988;**22**(7):832–836
- [19] Bernot MJ, Dodds WK, Gardner WS, McCarthy MJ, Sobolev D, Tank JL. Comparing denitrification estimates for a Texas estuary by using acetylene inhibition and membrane inlet mass spectrometry. *Applied and Environmental Microbiology*. 2003;**69**:5950–5956. DOI: 10.1128/AEM.69.10.5950-5956.2003
- [20] Torrentó C, Urmeneta J, Otero N, Soler A, Viñas M, Cama J. Enhanced denitrification in groundwater and sediments from a nitrate-contaminated aquifer after addition of pyrite. *Chemical Geology*. 2011;**287**(1-2):90–101. DOI: 10.1016/j.chemgeo.2011.06.002
- [21] Calderer M, Marti V, de Pablo J, Guivernau M, Prenafeta-Boldú FX, Viñan M. Effects of enhanced denitrification on hydrodynamics and microbial community structure in a soil column system. *Chemosphere*. 2014;**111**:112–119. DOI: 10.1016/j.chemosphere.2014.03.033
- [22] Gou H, Chen C, Lee DJ, Wang A, Gao D, Ren N. Coupled carbon, sulfur and nitrogen cycles of mixotrophic growth of *Pseudomonas* sp. C27 under denitrifying sulfide removal conditions. *Bioresource Technology*. 2014;**171**:120–126. DOI: 10.1016/j.biortech.2014.08.035



- [23] Xu G, Peng J, Feng C, Fang F, Chen S, Xu Y, Wang X. Evaluation of simultaneous autotrophic and heterotrophic processes and bacterial community structure analysis. *Applied Microbiology and Biotechnology*. 2015;**99**:6527–6536. DOI: 10.1007/s00253-015-6532-2
- [24] Szekeres S, Kiss I, Kalman M, Soares MI. Microbial population in a hydrogen-dependent denitrification reactor. *Water Research*. 2002;**36**(16):4088–4094. DOI: 10.1016/S0043-1354(02)00130-6
- [25] Kellerman C, Griebler C. *Thiobacillus thiophilus*, sp. nov., a chemolithoautotrophic, thiosulfate-oxidizing bacterium isolated from contaminated aquifer sediments. *International Journal of Systematic Evolution Microbiology*. 2009;**59**(3):583–588. DOI: 10.1099/ijs.0.002808-0
- [26] Junghare M, Patil Y, Schink B. Draft genome sequence of a nitrate-reducing, o-phthalate degrading bacterium, *Azoarcus* sp strain PA01T. *Standards in Genomic Sciences*. 2015;**10**:90. DOI:10.1186/s40793-015-0079-9
- [27] Hao R, Meng C, Li JJ. Impact of operation condition on the denitrifying bacterial community structure in a 3DBER-SAD reactor. *Journal of Industrial Microbiology and Biotechnology*. 2017;**44**:9–21. DOI:10.1007/s10295-016-1853-4
- [28] Osaka T, Yoshie S, Tsuneda S, Hirata A, Iwami N, Inamori W. Identification of acetate-or-methanol-assimilating bacteria under nitrate-reducing conditions by stable-isotope probing. *Microbial Ecology*. 2006;**52**(2):253–266. DOI: 10.1007/s00248-006-9071-7
- [29] Vasiliadou IA, Pavlou S, Vayenas DV. Dynamics of chemostat with three competitive hydrogen oxidizing denitrifying microbial populations and their efficiency for denitrification. *Ecological Modelling*. 2009;**220**(8):1169–1180. DOI: 10.1016/j.ecolmodel.2009.02.009
- [30] Mao Y, Xia Y, Zhang T. Characterization of *Thauera*-dominated hydrogen-oxidizing autotrophic denitrifying microbial communities by using high-throughput sequencing. *Bioresource Technology*. 2013;**128**:703–710. DOI: 10.1016/j.biortech.2012.10.106
- [31] Wang H, He Q, Chen D, Wei L, Zou Z, Zhou J, Yang K, Zhang H. Microbial community in a hydrogenotrophic denitrification reactor based on pyrosequencing. *Applied Microbiology and Biotechnology*. 2015;**99**(24):10829–10837. DOI: 10.1007/s00253-015-6929-y
- [32] Nakagawa S, Takai K, Inagaki F, Hirayama H, Nunoura T, Horikoshi K, Sako Y. Distribution phylogenetic diversity and physiological characteristics of epsilon-proteobacteria in a deep-sea hydrothermal field. *Environmental Microbiology*. 2005;**7**(10):1619–1632. DOI: 10.1111/j.1462-2920.2005.00856.x
- [33] Gevertz D, Telanj AV, Voordouw G, Jenneman GE. Isolation and characterization of strains CVO and FWKOB, two novel nitrate reducing, sulfide-oxidizing bacteria isolated from oil field brine. *Applied Environmental Microbiology*. 2000;**66**(6):2491–2501. DOI: 10.1128/AEM.66.6.2491-2501.2000
- [34] Yao S, Chen Q, Borthwick A. Enrichment and characterization of a bacteria consortium capable of heterotrophic nitrification and aerobic denitrification at low temperature. *Bioresource Technology*. 2013;**127**:151–157. DOI: 10.1016/j.biortech.2012.09.098

- [35] Liu BB, Mao YJ, Bergaust L, Bakken LR, Frostegard A. Strains in the genus *Thauera* exhibit remarkably different denitrification regulatory phenotypes. *Environmental Microbiology*. 2013;**15**(10):2816–2828. DOI: 10.1111/1462-2920.12142
- [36] Cuervo-López FM, Martínez-Hernández S, Texier A-C, Gómez J. Denitrification for waste water treatment. In: Cervantes F, editor. *Environmental Technologies to Treat Nitrogen Pollution. Principles and Engineering*. London, UK, International Water Association Publishing; 2009. pp. 41–65
- [37] Berks BC, Ferguson SJ, Moir JWB, Richardson DJ. Enzymes and associated electron transport system that catalyse the respiratory reduction of nitrogen oxides and oxyanions. *Biochemical et Biophysica Acta*. 1995;**1232**(3):97–133. DOI: 10.1016/0005-2728(95)00092-5
- [38] Mariotti A. Denitrification in groundwaters, principles and methods for its identification: A review. *Journal of Hydrology*. 1986;**88**:1–23
- [39] Betlach MR, Tiedje JM. Kinetic explanation for accumulation of nitrite, nitric oxide and nitrous oxide during bacterial denitrification. *Applied and Environmental Microbiology*. 1981;**42**:1074–1084
- [40] Song B, Palleroni NJ, Kerkhof LJ, Haggblom MM. Characterization of halobenzoate-degrading, denitrifying *Azoarcus* and *Thauera* isolates and description of *Thauera chlorobenzoica* sp.nov. *International Journal of Systematic and Evolutionary Microbiology*. 2001;**51**:589–602. DOI: 10.1099/00207713-51-2-589
- [41] Gudat GC, Singh J, Wharton DC. Cytochrome oxidase from *Pseudomonas aeruginosa*. Purification and some properties. *Biochimica et Biophysica Acta*. 1973;**292**(2):376–390. DOI: 10.1016/0005-2728(73)90044-3
- [42] Michalski W, Nicholas DJD. Molecular characterization of copper containing nitrite reductase from *Rhodopseudomonas sphaeroides* form sp. denitrificans. *Biochimica et Biophysica Acta*. 1985;**828**:130–137. DOI: 10.1016/0167-4838(86)90146-9
- [43] Carr GC, Ferguson SJ. The nitric oxide reductase of *Paracoccus denitrificans*. *Biochemical Journal*. 1990;**269**:423–429. DOI: 10.1042/bj2690423
- [44] Fujiwara T, Fukumori Y. Cytochrome cb-type nitric oxide reductase with cytochrome c oxidase activity from *Paracoccus denitrificans* ATCC35512. *Journal Bacteriology*. 1996;**178**:1866–1871. DOI: 10.1128/jb.178.7.1866-1871.1996
- [45] Berks BC, Baratta D, Richardson BJ, Ferguson SJ. Purification and characterization of a nitrous oxide reductase from *Thiosphaera pantotropha*. *European Journal of Biochemical*. 1993;**212**:467–476. DOI: 10.1111/j.1432-1033.1993.tb17683.x
- [46] Saleh-Lakha S, Shannon KE, Henderson SL, Goyer C, Trevors KT, Zebarth BJ, Burton DL. Effect of pH and temperature on denitrification gene expression and activity in *Pseudomonas mandelii*. *Applied and Environmental Microbiology*. 2009;**75**:3903–3911. DOI: 10.1128/AEM.00080-09

- [47] Elefsiniotis P, Li D. The effect of temperature and carbon source on denitrification using volatile fatty acids. *Biochemical Engineering Journal*. 2006;**28**:148–155. DOI: 10.1016/j.bej.2005.10.004
- [48] Cervantes F, Monroy O, Gomez J. Influence of ammonium on the performance of a denitrifying culture under heterotrophic conditions. *Applied Biochemistry and Biotechnology*. 1999;**81**:13–21. DOI: 10.1385/ABAB:81:1:13
- [49] Tiedje JM. Ecology of denitrification and dissimilation nitrate to ammonium. In: Zender AJB, editor. *Environmental Microbiology of Anaerobes*. New York: Wiley; 1988. pp. 179–244
- [50] Hernandez D, Rowe JJ. Oxygen regulation of nitrate uptake in denitrifying *Pseudomonas aeruginosa*. *Applied and Environmental Microbiology*. 1987;**53**:745–750. DOI: 0099-2240/87/040745-06\$02.00/0
- [51] Ferguson SJ. Denitrification and its control. *Antonie van Leeuwenhoek*. 1994;**66**:89–110. DOI: 10.1007/BF00871634
- [52] Dobbie KE, Smith KA. The effects of temperature, water-filled pore space and land use on N<sub>2</sub>O emissions from an imperfectly drained gleysol. *European Journal of Soil Science*. 2001;**52**(4):667–673. DOI: 10.1046/j.1365-2389.2001.00395.x
- [53] Knowles R. Denitrification. *Microbial Reviews*. 2001;**46**:43–70. DOI: 0146-0749/82/010043-28\$02.00/0
- [54] Thomsen JK, Geest T, Cox RP. Mass spectrometric studies of the effect of pH on the accumulation of intermediates in denitrification by *Paracoccus denitrificans*. *Applied and Environmental Microbiology*. 1994;**60**:536–541
- [55] Li YM, Li J, Zheng GH, Luan J-F, Fu QS, Gu G-W. Effects of the COD/NO<sub>3</sub><sup>-</sup>-N ratio and pH on the accumulation of denitrification intermediates with available pyridine as a sole electron donor and carbon source. *Environmental Technology*. 2008;**29**:1297–1306. DOI: 10.1080/09593330802379672
- [56] Bisailon A, Beaudet R, Lépine F, Déziel E, Villemur R. Identification and characterization of novel CprA reductive dehalogenase specific to highly chlorinated phenols from *Desulfotobacterium hafniense* strain PCP-1. *Applied Environmental Microbiology*. 2010;**76**(22):7536–7540. DOI: 10.1128/AEM.01362-10
- [57] Field JA, Sierra-Alvarez R. Microbial degradation of chlorinated phenols. *Reviews in Environmental Science and Biotechnology*. 2008 **7**(3):211–241. DOI: 10.1007/s11157-007-9124-5
- [58] Chang C-C, Tseng SK, Chang C-C, Ho C-H. Reductive dechlorination of 2-chlorophenol in a hydrogenotrophic, gas permeable, silicone membrane bioreactor. *Bioresource Technology*. 2003;**90**:323–328. DOI: 10.1016/S0960-8524(03)00149-4
- [59] Yu H-Y, Wang Y-K, Chen P-C, Li F-B, Chen M-J, Hu M, Ouyang X. Effect of nitrate addition on reductive transformation of pentachlorophenol in paddy soil relation to iron

- (III) reduction. *Journal of Environmental Management*. 2014;**132**:42–48. DOI: 10.1016/j.jenvman.2013.10.020
- [60] Häggblom MM, Rivera MD, Young LY. Influence of alternative electron acceptors on the anaerobic biodegradability of chlorinated phenols and benzoic acids. *Applied and Environmental Microbiology*. 1993;**59**:1162–1167. DOI: 0099-2240/93/041162-06\$02.00
- [61] Moussavi G, Ghodrati S, Mohseni-Bandpaei A. The biodegradation and COD removal of 2-chlorophenol in a granular anoxic baffled reactor. *Journal of Biotechnology*. 2014;**184**:111–117. DOI: 10.1016/j.jbiotec.2014.05.010
- [62] Wang X, Xing L, Qui T, Han M. Simultaneous removal of nitrate and pentachlorophenol from simulated groundwater using a biodenitrification reactor packed with corncob. *Environmental Science and Pollution*. 2013;**20**(4):2236–2243. DOI: 10.1007/s11356-012-1092-9
- [63] D'Angelo EM, Reddy KR. Aerobic and anaerobic transformation of pentachlorophenol in wetlands soils. *Soil Science Society of America Journal*. 2000;**64**(3):933–943. DOI:10.2136/sssaj2000.643933x
- [64] Jianlong W, Horan N, Stentiford ED, Yi Q. Anaerobic degradation of 4-chlorophenol under denitrifying conditions with nitrate as the only electron acceptor. *Toxicological and Environmental Chemistry*. 2001;**80**:209–216. DOI:10.1080/02772240109359011
- [65] Bae H, Yamagishi T, Suwa Y. Developing and sustaining 3-chlorophenol-degrading populations in up-flow column reactors circum-denitrifying conditions. *Applied Microbiology and Biotechnology*. 2002b;**59**:118–124. DOI: 10.1007/s00253-002-0974-z
- [66] Hu Z, Ferraina RA, Ericson JF, Smets BF. Effect of long-term exposure, biogenic substrate presence, and electron acceptor conditions on the biodegradation of multiple substituted benzoates and phenolates. *Water Research*. 2005;**39**:3501–3510. DOI: 10.1016/j.watres.2005.06.009
- [67] Farrel A, Quilty B. The enhancement of 2-chlorophenol degradation by a mixed microbial community when augmented with *Pseudomonas putida* CP1. *Water Research*. 2002;**36**:2443–2450. DOI: 10.1016/S0043-1354(01)00481-X
- [68] Fakhruddin ANM, Quilty B. The influence of glucose and fructose on the degradation of 2-chlorophenol by *Pseudomonas putida*. *World Journal of Microbiology and Biotechnology*. 2005;**21**(8):1541–1548. DOI: 10.1007/s11274-005-7580-z
- [69] Perkins PS, Komisar SJ, Puhakka JA, Ferguson FJ. Effects of electron donors and inhibitors on reductive dechlorination of 2,4,6-trichlorophenol. *Water Research*. 1994;**28**:2101–2107. DOI: 10.1016/0043-1354(94)90020-5
- [70] Chaudhuri BK, Wiesmann U. Enhanced anaerobic degradation of benzene by enrichment of mixed microbial culture and optimization of culture medium. *Applied Microbiology and Biotechnology*. 1995;**43**:178–187. DOI: 10.1007/BF00170641

- [71] Martínez-Hernández S, Olguín EJ, Gómez J, Cuervo-López FM. Acetate enhances the specific consumption rate of toluene under denitrifying conditions. *Archives of Environmental Contamination and Toxicology*. 2009;**57**:679–687. DOI: 10.1007/s00244-009-9321-z
- [72] Puyol D, Mohedano AF, Sanz JL, Rodríguez JJ. Comparison of UASB and EGSB performance on the anaerobic biodegradation of 2,4-dichlorophenol. *Chemosphere*. 2009;**76** (9):1192–1198. DOI: 10.1016/j.chemosphere.2009.06.015
- [73] Martínez-Gutiérrez E, González-Márquez H, Martínez-Hernández S, Texier A-C, Cuervo-López FM, Gómez J. Effect of phenol and acetate addition on 2-chlorophenol consumption by a denitrifying sludge. *Environmental Technology*. 2012;**33**:1375–1382. DOI: 10.1080/09593330.2011.627882
- [74] Daghighi M, Vaipoulou E, Patil SA, Suárez-Suárez A, Head IM, Franzetti A, Rabaey K. Anodes stimulate anaerobic toluene degradation via sulfur cycling in marine sediments. *Applied and Environmental Microbiology*. 2016;**82**:297–307. DOI: 10.1128/AEM.02250-15
- [75] Yeruva DK, Jukuri S, Velvizhi G, Kumar AN, Swamy YV. Integrating sequencing batch reactor with bio-electrochemical treatment for augmenting remediation efficiency of complex petrochemical wastewater. *Bioresource Technology*. 2015;**188**:32–43. DOI: 10.1016/j.biortech.2015.02.014
- [76] Fontmorin J-M, Fourcade F, Geneste F, Floner D, Huguet S, Amrane A. Combined process for 2,4-cychlorophenoxyacetic acid treatment coupling of an electrochemical system with biological treatment. *Biochemical Engineering Journal*. 2013;**70**:17–22. DOI: 10.1016/j.bej.2012.09.015
- [77] Dixit A, Tirpude AJ, Mungray AK, Chakraborty M. Degradation of 2,4 DCP by sequential biological-advanced oxidation process using UASB an UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub>. *Desalination*. 2011;**272**:265–269. DOI: 10.1016/j.desal.2011.01.035
- [78] Arellano-González MA, González I, Texier A-C. Mineralization of 2-chlorophenol by sequential electrochemical reductive dechlorination and biological processes. *Journal of Hazardous Materials*. 2016;**314**:181–187. DOI: 10.1016/j.jhazmat.2016.04.048