Advances in ozonation and biodegradation processes to enhance chlorophenol abatement in multisubstrate wastewaters: a review

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Chlorophenolic pollutants are considered a serious environmental threat when disposed without proper treatment. Moreover, increasingly stringent regulations towards effluent quality result in a growing interest in upgrading conventional wastewater treatment to reduce pollutant concentrations to acceptable levels. Among the wide range of treatment options, biological and ozone-based oxidation processes are already well defined for the degradation of chlorophenolic compounds. The main objective of this review is to highlight relevant knowledge gaps and research needs on chlorophenol degradation by applying biological and/or ozone-based oxidation techniques. The main research trends towards chlorophenol biodegradation are the use of specific cultures adapted to the pollutant and co-metabolic biodegradation. More recently, novel biological processes such as biomass immobilization and granular activated sludge were reported. These techniques possess the ability to protect the microorganisms from higher toxic pollutant concentrations. Only a few authors reported the use of mixed cultures and the effects of acclimated biomass to enhance the biodegradation process. However, conventional biological processes do not always achieve satisfactory results, opening the possibility for ozone-based oxidation technologies to degrade the refractory compounds into more biodegradable intermediates. The research objectives in many studies on chlorophenol ozonation consider the degradation of single pollutants and/or the enhancement of the oxidative potential using catalysts or UV light. Only a few attempts were reported investigating chlorophenol degradation in multiple substrate solutions. Also, the need for an additional toxicity and/or biodegradability assessment using microorganisms representative for activated sludge is clear, the more when assessment of effects on a totally different microbial community is desired.

Water impact

Chlorophenol contamination is a serious threat to receiving water bodies. Hence, the treatment of chlorophenolic wastewater is critical. Both biological and ozone-based oxidation processes are well defined for chlorophenol degradation. By highlighting the state-of-the-art, the applicability of these techniques is shown. Furthermore, novel trends including immobilized biomass or the need for ozone degradation studies in multiple substrate solutions were identified.

1. Introduction

Because of industrial, agricultural, and domestic activities, significant amounts of toxic and hazardous pollutants are emitted to the environment. Most of these pollutants possess xenobiotic and recalcitrant properties, tend to bioaccumulate and show high persistence in the environment. Increased knowledge on the consequences of water pollution, diminishing water resources and industrial development have created the need for regulations.¹ The European Water Framework Directive (2000/60/EC) imposes EU member states to prevent and reduce pollution, to promote sustainable water usage and to protect ecosystems. A list of priority substances was proposed (2455/2001/EC) and this was updated in subsequent Directive (2008/105/EC; 2013/39/EC). Furthermore, the

Environmental Protection Agency also published a similar list of 129 priority pollutants including several chlorophenolic pollutants.²

As a consequence, both effluent quality and quantity are subjected to increasingly stringent regulations, resulting in a growing interest in wastewater treatment to reduce pollutant concentrations to acceptable levels. However, the selection of the best treatment technology is complex. According to Oller *et al.*, the choice to combine one or more wastewater treatment processes depends on the composition of the wastewater, the effluent standards to be met and the most effective treatment with the lowest reasonable cost.³ Despite their widespread application, conventional wastewater treatment technologies, such as biological processes, do not always achieve satisfactory purification results. The presence of toxic and/or recalcitrant pollutants can affect the biodegradation process, resulting in the discharge of harmful substances into rivers, lakes, soil and groundwater, thereby creating a serious threat to ecosystems.

A classification of the pollutants affecting the biodegradation is proposed by Alvares *et al.* and Tabrizi and Mehrvar, i.e., recalcitrant and inhibitory pollutants.^{1,4} Recalcitrant pollutants

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are considered to be resistant to (aerobic) microbial degradation under favourable environmental conditions, whereas inhibitory pollutants, potentially biodegradable, present some degree of toxicity or activity inhibition to the bioculture.⁴⁻⁵ However, clear classification is not always possible, e.g., since recalcitrant pollutants may accumulate, resulting in toxic concentrations. The extent to which the biological process is affected depends on the concentration, solubility and the molecular complexity (e.g. chemical structure and number of substituents) of the pollutant.⁵ Because of the shortcomings of biological treatments, recalcitrant pollutants are discharged and may accumulate in receiving waters. For this reason, the optimal functioning of ecosystems may be hindered.

In particular, chlorophenols (CP) pose a serious threat because of their widespread occurrence in soils, sediments, surface and ground water.⁶ These pollutants and their derivatives are mutagenic, carcinogenic and immunogenic for humans and other organisms.⁷⁻⁸ Pollutants such as 2,4,6-trichlorophenol (2,4,6-TCP), 2,4,5-trichlorophenol (2,4,5-TCP) and pentachlorophenol (PCP) are classified by the International Agency for Research on Cancers (IARC) as possibly carcinogenic to humans.⁹ To decrease the toxicity to receiving water bodies, the treatment of chlorophenolic wastewater is critical. Several treatment technologies have already been investigated for the removal of chlorophenols from wastewater, such as activated carbon adsorption, volatilization, solvent extraction, chemical oxidation and aerobic/anaerobic biodegradation.¹⁰⁻¹⁴ Absorption and adsorption methods are based on a phase transfer of the chlorophenolic chemicals, imposing further treatment of the solvent or adsorbent. To achieve degradation of the pollutants (instead of concentrating them in a different phase), oxidative techniques are of great interest. Specific microbial cultures utilize some chlorophenols as their sole carbon and energy source under aerobic conditions.¹⁵⁻¹⁶ Furthermore, co-metabolic biodegradation of chlorophenolic pollutants can be successfully enhanced in the presence of a growth substrate such as glucose or phenol.¹⁷⁻¹⁸ Co-metabolism is the biological transformation of a non-growth substrate by non-specific enzymes of bacteria, which the synthesis of these enzymes is only induced by a growth-substrate.¹⁹ Nevertheless, the biodegradation of chlorophenols is often very difficult because of the specific conditions or the long reaction time required.

In this context, Advanced Oxidation Processes, and ozone-based techniques in particular, are considered an appropriate tool for the conversion of refractory compounds into more biodegradable intermediates.²⁰ Indeed, because of the very high reaction rate of phenolate ions with ozone, ozonation appears to be a promising oxidation technique for the degradation of chlorophenolic pollutants.²¹ The dissociation of phenolic pollutants into phenolate ions occurs at pH values higher than the dissociation constant. The dissociation constants (pKa) of some chlorophenols are listed in Table 1. At these high pH values, the production of hydroxyl radicals is favoured. However, direct ozonation can also be important when the concentration of highly reactive species with molecular ozone is sufficiently high. Moreover, according to Benítez *et al.* and Hong and Zeng, the

secondary hydroxyl radicals are likely to be involved in the subsequent degradation of intermediates.²²⁻²³

This review article critically discusses the developments on biological and ozone-based oxidation of chlorophenolic pollutants being reported in the scientific literature in the last two decades. An overview of experimental studies is listed to summarize degradation pathways and recent innovations in reactor design, configuration strategies and process development. Also, different methodologies regarding biodegradability and toxicity assessment are discussed. The objective is to emphasize relevant knowledge gaps and research needs on chlorophenol degradation by applying biological and/or ozone-based oxidation techniques.

2. Characteristics of chlorophenolic pollutants

Chlorophenols are derivatives of phenol, which contain one or more covalently bound chlorine atoms. The common fragment in phenolic compounds is the hydroxyl group attached to an aromatic sp²-hybridized carbon atom, forming a strongly activating substituent.²⁴ The group of chlorophenols contains 19 different congeners including mono-, di-, tri-, tetra- and pentachlorophenol. Among these, mainly seven chlorophenols are applied in industry, i.e., monochlorophenol (MCP), 2,4-dichlorophenol (2,4-DCP), 2,4,5-TCP, 2,4,6-TCP, 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP), 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP) and PCP.⁶ Their physical properties are listed in Table 1.

Because both the hydroxyl group and aromatic ring can react via both electrophilic and nucleophilic substitution, chlorophenols are readily oxidizable. Hence, these chemical structures are versatile intermediates in chemical synthesis. For example, 2-CP, 2,4-DCP and 2,4,6-TCP are used as intermediates in the production of higher chlorophenols, such as TeCP and PCP, and of chloride-based herbicides, such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-(2,4 dichlorophenoxy)propionic acid.²⁵⁻²⁶ Moreover, 2,4,6-TCP is used in the preparation of flame retardants.²⁷ Because of their antimicrobial properties, chlorophenolic chemicals are primarily used in pulp and paper, textile, leather tanning and wood bleaching industries.^{6, 28-29} Also, their presence in wastewater originating from oil refining, petrochemicals and coking plants has been reported.³⁰⁻³¹ Tobajas *et al.* report the use of phenols and chlorophenols in many industrial processes such as the production of resins, nylon, plastics, antioxidants, lubricant additives, textile, dyes or explosives.³² Their presence in industrial wastewater ranges from one to several hundred mg/L.³³ Applications as herbicides, insecticides, fungicides and wood preservatives are reported.³⁴ They are also observed in landfill leachates or as byproducts of the disinfection by chlorination.^{6, 35-36}

Table 1: Literature data on octanol/water partition coefficient (KOW), dissociation constant (pKa) and toxicity based on in vitro cytotoxicity and *Daphnia Magna* inhibition

Pollutant	Formula	CAS Number	Molecular weight ^a [g/mol]	Melting point ^a [°C]	Boiling point ^a [°C]	Solubility ^a [g/L]	Log K _{ow} ^b	pK _a ^b	Cytotoxicity Log(1/LC ₅₀) ^c	<i>Daphnia magna</i> inhibition Log(1/LC ₅₀) ^d
2-CP	C ₆ H ₅ Cl O	95-57-8	128.6	9.4	175	20.00	2.12-2.17	8.3-8.6	2.63	3.86
3-CP	C ₆ H ₅ Cl O	108-43-0	128.6	32.6	214	22.00	2.48-2.50	8.8-9.1		3.91
4-CP	C ₆ H ₅ Cl O	106-48-9	128.6	42.8	220	28.00	2.35-2.44	9.1-9.4	2.68	4.20
2,3-DCP	C ₆ H ₄ Cl ₂ O	576-24-9	163.0	58.0		8.22	3.15-3.19	6.4-7.8		4.50
2,4-DCP	C ₆ H ₄ Cl ₂ O	120-83-2	163.0	45.0	210	4.90	2.75-3.30	7.5-8.1	3.11	4.78
2,5-DCP	C ₆ H ₄ Cl ₂ O	583-78-8	163.0	59.0	211		3.20-3.24	6.4-7.5		4.56
2,6-DCP	C ₆ H ₄ Cl ₂ O	87-65-0	163.0	68.5	220	2.63	2.57-2.86	6.7-7.8	3.06	4.24
3,4-DCP	C ₆ H ₄ Cl ₂ O	95-77-2	163.0	68.0	253		3.13-3.44	7.4-8.7	3.23	4.77
3,5-DCP	C ₆ H ₄ Cl ₂ O	591-35-5	163.0	68.0	233		2.57-3.56	6.9-8.3		4.89
2,3,4-TCP	C ₆ H ₃ Cl ₃ O	15950-66-0	197.5	83.5			3.49-4.07	6.5-7.7	3.42	4.95
2,3,5-TCP	C ₆ H ₃ Cl ₃ O	933-78-8	197.5	62.0	248		3.84-4.56	6.8-7.4	3.62	4.94
2,3,6-TCP	C ₆ H ₃ Cl ₃ O	933-75-4	197.5	58.0			3.88	6.0-7.1		4.43
2,4,5-TCP	C ₆ H ₃ Cl ₃ O	95-95-4	197.5	69.0	247	1.00	3.72-4.10	7.0-7.7		4.98
2,4,6-TCP	C ₆ H ₃ Cl ₃ O	88-06-2	197.5	69.0	246	0.50	3.60-4.05	6.0-7.4	3.42	4.56
3,4,5-TCP	C ₆ H ₃ Cl ₃ O	609-19-8	197.5	101.0	275		4.01-4.39	7.7-7.8		5.35

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2,3,4,5- TeCP	C ₆ H ₂ Cl ₄ O	4901-51-3	231.9	116.5			4.21-5.16	6.2-7.0		5.12
2,3,4,6- TeCP	C ₆ H ₂ Cl ₄ O	935-95-5	231.9	70.0	150	0.17	4.10-4.81	5.3-6.6		4.93
2,3,5,6- TeCP	C ₆ H ₂ Cl ₄ O	58-90-2	231.9	115.0			3.88-4.92	5.2-5.5	3.87	5.01
PCP	C ₆ Cl ₅ O H	25167-83-3	266.3	174	310	0.01	5.01-5.86	4.7-4.9	4.03	5.55

^a From Lide³⁷^b From Czaplicka⁶^c From Liu et al.³⁸; Cytotoxicity measured by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltriazolium bromide reduction assay on mouse connective tissue fibroblast L929 cells (24 h exposure)^d From Pera-Titus et al.³⁹; the *Daphnia magna* were all > 72h old. The water hardness was 200 mg/L as CaCO₃, dissolved oxygen > 2.27 mg/L, pH 7.8–8.2 and temperature 20 °C (24 h exposure)

Furthermore, the *ortho*-substituted molecules are generally lower toxicity than the *meta*- and *para*-ones. According Grimwood and Mascarenhas, the *ortho*-substituted chlorophenols shields off the hydroxyl group, which interacts with the active sites in aquatic organisms.⁴³ So, the interaction of an *ortho*-substituted chlorophenol with microorganisms is partially blocked, and the microorganisms experience less toxicity effects. However, the dissociation of chlorophenolic molecules affects the bioaccumulation, and also the toxicity. Only limited changes in toxicity will be observed at a pH below the dissociation constant (pKa), but a sharp decrease of toxicity when pH is higher than pKa.⁴³ So, the toxicity increases by the presence of non-dissociated forms.³⁹

3. Biodegradation of chlorophenolic pollutants

3.1. Degradation pathways

The most critical step in the biodegradation of chlorophenols is the cleavage of the carbon-halogen bond, which occurs via the direct removal of the halogen from the aromatic ring (as initial degradation step) or after oxygenative ring cleavage (from chlorinated aliphatic intermediates).⁴⁴ The cleavage of the carbon halogen bond is confirmed by earlier studies indicating that chlorinated phenols are degraded to chlorine free end-products.^{15,45-49}

Depending on the degree of chlorination, two main mechanisms are reported as the initial reaction step to degrade chlorophenolic pollutants by aerobic bacteria:^{15,50}

- 1. The chlorocatechol pathway.** The initial attack by monooxygenase, yielding chlorocatechols as first intermediates, occurs mainly with lower chlorinated phenolic pollutants (from 1 to 2 chlorine substituents). Farrel and Quilty reported the transformation of 2-CP and 3-CP into 3-chlorocatechol, whereas the production of 4-chlorocatechol was observed during biotreatment of 4-CP (Figure 1).¹⁵ These intermediate chlorocatechols were further subject to ring cleavage.
- 2. The hydroquinone pathway.** The conversion of higher chlorinated chlorophenols (from 3 to 5 chlorine substituents) results in the production of chlorohydroquinones as first intermediates. Chlorines are progressively removed from the ring prior to ring cleavage. The degradation pathway of 2,4,5-trichlorophenoxyacetic

acid (2,4,5-T) via hydroquinone is proposed by Solyanikova and Golovleva (Figure 2).⁵¹

The cleavage of the aromatic ring occurs according to the *ortho*- or *meta*-pathway. Farrel and Quilty mentioned a modified *ortho*-pathway specific for chlorinated aromatic pollutants, which is necessary to achieve a complete degradation.¹⁵

Figure 1: suggested biodegradation pathway of 2-CP in aerobic circumstances⁵²

This cleavage pathway is catalysed by the enzyme catechol-1,2-dioxygenase and results in the release of the chloride atom. However, the *meta*-pathway is identified as an unproductive route for chlorophenol degradation caused by the production of dead-end products, such as 5-chloro-2-hydroxyruconic semialdehyde.⁵³

Direct elimination of chlorine substituents from the aromatic ring occurs via the replacement with hydrogen atoms, which requires a reducing agent (reductive dechlorination) and is significant in anaerobic environments.⁴⁴

Annachhatre and Gheewala reported reductive dehalogenation as the initial reaction step for the biodegradation of chlorinated phenols under anaerobic conditions.⁴² Puyol *et al.* concluded that 4-CP was the main chlorinated intermediate product for 2,4,6-TCP and 2,4-DCP biodegradation, suggesting that the *ortho* dechlorination is the predominant degradation pathway.⁴⁸ Also,

Figure 2: Pathway of 2,4,5-T conversion via hydroxyquinol (tftAB and tftCD encode 2,4,5-T oxygenase and monooxygenase, respectively; tftG encodes enzyme responsible for dechlorination of 5-chlorohydroxyquinol)⁵¹

1 Lv *et al.* concluded that reductive dechlorination was the
 2 predominant biotransformation process of PCP under oxygen-
 3 limited conditions.⁵⁴ To achieve a complete mineralization,
 4 reductive dechlorination can be followed by carboxylation, ring
 5 fission, acetogenesis and methanogenesis.⁵⁵ Nevertheless, Puy-
 6 *et al.* reported the limitations for the aerobic biodegradation of
 7 monochlorinated phenolics.⁵⁶
 8 Moreover, Yang *et al.* tested the anaerobic biodegradability of
 9 46 aromatic compounds, including 4-CP which was classified as
 10 poorly biodegradable.⁵⁷

11 3.2. Single source versus co-metabolic 12 degradation

13 Several reports, summarized in Table 2, are available in which
 14 chlorophenols were used by specific isolated bacteria as a single
 15 carbon and energy source. *Pseudomonas stutzeri* was able to
 16 grow and utilize PCP as an energy source, even at high
 17 concentrations, resulting in a complete degradation of the
 18 pollutant after 120 h.⁵⁸ Karn *et al.* investigated *Bacillus*
 19 *megaterium*, *B. pumilus* and *B. thuringensis*, isolated from pulp
 20 and paper mill secondary sludge.⁵⁰ All three isolated strains
 21 achieved a complete removal of 100 mg/L PCP within 168 h.
 22 During growth, chloride was released to the medium because of
 23 the consumption of PCP. *Arthrobacter chlorophenolicus*
 24 achieved 4-CP biodegradation efficiencies between 70% and
 25 99.8%, depending on the conditions.⁵⁹ Carvalho *et al.*
 26 investigated the fungus *Mucor plumbeus* for the degradation of
 27 PCP.⁶⁰ The biodegradation of 100 mg/L 4-CP by *Pseudomonas*
 28 *sp.* was achieved after 40 h with a lag phase for the biomass
 29 activity of 10 h.⁶¹ *Penicillium sp.* was used by Yan *et al.* for the
 30 biodegradation of DCMX in high saline wastewater.⁶² The
 31 degradation efficiency decreased significantly at high salt
 32 concentrations, i.e., 4%, mainly as NaCl. Shi *et al.* screened the
 33 feasibility for phytoremediation of 2,4-DCP using four *Salt-*
 34 *matsudana* clones (plant enzymes).⁴⁰ Removal efficiencies
 35 between 52.2% and 73.7% were reported. However, the removal
 36 efficiency decreased significantly by increasing the initial
 37 concentration of 2,4 DCP.

38 For select bacteria, the sole presence of chlorophenols is
 39 insufficient to support microbial growth because of their low
 40 energy content.⁶³ In the presence of other carbon sources, the
 41 degradation of chlorophenols seems to proceed easier.³² An
 42 overview of studies regarding co-metabolic biodegradation of
 43 chlorophenols by pure isolated cultures is included in Table 3.
 44 The presence of easily degradable carbon sources stimulates the
 45 growth of the microbial population, which in turn enhances the
 46 biodegradation of target pollutants and reduces the toxicity and
 47 growth inhibition.²⁶ Even the dosing mode of the growth
 48 substrate influences the co-metabolic degradation. Wang *et al.*
 49 applied three dosing modes of sodium acetate, i.e., one-step
 50 pulse, evenly distributed pulse, and stepwise declining pulse,
 51 which the last two showed an enhancement of the PCP
 52 degradation rate.⁶⁴

53 Several findings suggest the co-metabolic degradation of
 54 chlorophenols by addition of glucose, peptone, molasses,
 55 sucrose, maltose, acetate, formate or propionate.^{13,26,32,52,65}

Nevertheless, the type of growth substrate is determinative of the
 chlorophenol degradation. Majumder & Gupta achieved higher
 chlorophenol removal efficiencies by using glucose or methanol
 instead of formate or propionate.⁶⁶

Even phenol is claimed to be a good growth substrate because of
 its similar chemical structure and lower toxicity.^{19,32,61,76} Tobajas
et al. mentioned the danger of diauxic growth, which implies the
 inhibition of the consumption of one growth substrate by the
 presence of another substrate.³² However, co-metabolic
 degradation of chlorophenols results in the enhancement of the
 degradation rate and the reaction time. Wang *et al.* described the
 degradation of 2,4-DCP in the presence of phenol by *Bacillus*
insolitus cultivated in suspended and immobilized form and
 concluded that only at low 2,4-DCP concentrations immobilized
 cells showed a higher removal of 2,4-DCP than suspended
 ones.⁷⁷ At higher concentrations, similar removal efficiencies
 were observed until the growth was fully inhibited by too high
 chlorophenol concentrations. The degradation of 4-CP in a
 mixture with phenol and sodium glutamate by *Pseudomonas*
putida was investigated by Wang *et al.*¹⁷ The biphasic growth
 pattern on the ternary substrate mixture showed two exponential
 growth phases separated by an intermediate lag phase. The same
 bacteria were used to degrade a mixture of 600 mg/L phenol and
 120 mg/L 4-CP.¹⁸ The suspended cells were not able to degrade
 either of both substrates. However, a complete transformation at
 higher concentrations of both substrates (1000 mg/L) was
 achieved within 45 h by using an immobilized cell hollow fiber
 membrane reactor. The simultaneous biodegradation of phenol
 and 4-CP was observed by Wang *et al.*⁷⁸ However, the
 degradation of 4-CP increased only after the complete utilization
 of phenol. Tobajas *et al.* concluded that the simultaneous
 addition of phenol or glucose significantly reduces the minimum
 time required for complete 4-CP degradation by *Comamonas*
testosterone.³² The treatment of a complex mixture containing
 500 mg/L PCP and 200 mg/L Cr⁶⁺ by *Bacillus cereus* was
 investigated by Tripathi and Garg.⁷² The bioreduction of Cr⁶⁺ to
 insoluble Cr³⁺ and simultaneous dechlorination of PCP was
 achieved after the addition of an extra carbon source (glucose,
 maltose and sucrose) and nitrogen source (ammonium chloride,
 ammonium nitrate or urea). Tetra-, tri- and dichlorophenols and
 6-chlorohydroxyquinol were detected as PCP dechlorination
 products. A decrease in reaction time by 18 h for the treatment
 of 4-CP by *Pseudomonas sp.* was achieved through the addition
 of phenol.⁶¹ Nevertheless, the degradation time of phenol was
 extended by 11 h when 4-CP was also present in the wastewater.
 The authors also observed a lag phase in biomass activity before
 any pollutant was degraded. Patel and Kumar used peptone as
 growth substrate for the degradation of 2,4-DCP by *B.*
endophyticus and observed a complete 2,4-DCP degradation in
 the range of 25-50 mg/L 2,4-DCP and between 75% and 80%
 degradation in the range of 100 mg/L 2,4-DCP to 400 mg/L 2,4-
 DCP.⁷⁴ Nowak *et al.* used *Stenotrophomonas maltophilia* for the
 biodegradation of monochlorophenols and concluded that the co-
 metabolic degradation efficiency depended on the earlier
 exposure to phenol and the position of the chlorine substituent in
 the aromatic ring.⁷⁹

In addition to more common co-metabolites, saponic extracts of

Table 2: The single source biodegradation of chlorophenolic pollutants by using pure isolated cultures

Reference	Target Pollutant(s)	Conditions	Highlights of the work
<i>Using suspended-growth biomass</i>			
50	PCP (50-600 mg/L)	Inoculum: <i>Bacillus megaterium</i> CL3, <i>B. pumilus</i> CL5 and <i>B. thuringensis</i> CL11 (Pulp and paper mill secondary sludge) Cultivation: 50 mg/L PCP (48 h, 37 °C) Experiments: - pH = 7.5, 8.5 and 9.5 - Temperature = 25-37 °C	All three isolates were able to grow and utilize PCP as an energy source. Complete removal of 100 mg/L PCP by all three isolates within 168 h. Chloride was released in the medium during growth. Temperature and pH affects both biomass growth and PCP degradation.
58	PCP (50-600 mg/L)	Inoculum: <i>Pseudomonas stutzeri</i> CL7 (Pulp and paper mill secondary sludge) Cultivation: 50 mg/L PCP (48 h, 37 °C) Experiments: - pH = 7.5, 8.5 and 9.5 - Temperature = 25-37 °C	<i>P. stutzeri</i> CL7 was able to grow and utilize PCP as an energy source, even at high concentrations. Degradation efficiencies of 100 mg/L PCP: 60%, >90% and 100% after 24 h, 72 h and 120 h, respectively (simultaneous release of chloride) Higher growth and degradation efficiency at higher temperature.
59	4-CP (300 mg/L)	Inoculum: <i>Arthrobacter chlorophenolicus</i> A6 Cultivation: 150 mg/L 4-CP (48 h, 28 °C)	4-CP in the media was utilized mainly for biomass growth. 4-CP biodegradation efficiency: 70-99.8% Accumulation of SO ₄ ²⁻ and NO ₃ ⁻ as well as PO ₄ ³⁻ ions are inhibitory to the reductive dechlorination step. However, PO ₄ ³⁻ had a strong positive impact on microbial degradation of aromatics because of its buffering action.
60	PCP (4 mg/L)	Inoculum: <i>Mucor plumbeus</i> Bonord (DSM 16513; Fungi) Cultivation: 4 mg/L PCP (pH 6, 27 °C, 72 h)	Residual amount of PCP: 1% The results indicated PCP uncoupling of oxidative phosphorylation in mitochondria.
62	DCMX ⁽¹⁾ (5-60 mg/L) in high saline wastewater	Inoculum: <i>Penicillium sp.</i> yz11-22N2 Cultivation: 10 g/L glucose (pH 7, 30 °C) Experiments: - Salinity = 2.0 – 4.0% - pH = 5.5 – 8.0 - Exposure time = 12 days	The maximum DCMX removal capacity was obtained under acidic conditions (optimal at pH 6.0). The growth of <i>Penicillium sp.</i> on glucose was significantly decreased when DCMX was present (about 41% reduction of the average size of mycelium). At 2.0% and 3.0% salinity, the final removal rate of DCMX was significantly higher compared to 4.0% salinity. Furthermore, the toxic effect of DCMX was more obvious at high salinity. At high DCMX concentrations of 40 mg/L and 60 mg/L, strong inhibition effects on the microorganisms were observed, resulting in a decline of the removal rate.

			Degree of importance of the investigated parameters: salinity>temperature>pH.
<i>Using attached-growth biomass</i>			
40	2,4-DCP (10-30 mg/L)	Inoculum: Plant enzymes <i>Salix matsudana</i> clones (8, 10, 18 and 22) Cultivation: Hoagland solution (3 months) Experiments: - 16 h at 25-35 °C during the day, 8 h at 10-18 °C during the night - Exposure time = 96 h - pH = 5.6	The clones were only to a minor extent inhibited by 2,4-DCP levels up to 10 mg/L. 2,4-DCP removal efficiency: 52.2-73.7%
80	BISPHENOL A (10 mg/L) 2,4-DCP (10 mg/L) TRICLOSAN (10 mg/L)	Inoculum: enzyme Laccase (<i>p</i> -diphenol:dioxygen oxidoreductase, PDLLA) Immobilization: Laccase-carrying electrospun fibrous membranes enhanced by short and acid-treated multi-walled carbon nanotubes Experiments: - 2 mg immobilized laccase to 30 mL phenolic aqueous solution - Exposure time = 5 h - pH = 7.0 - Temperature = 25 °C	Because of the immobilization of laccase in the original fibers, the degradation efficiencies of Bisphenol A and 2,4-DCP decreased. However, Triclosan was adsorbed rapidly around the active sites of laccase, resulting in the increase of its degradation efficiency. Because of the modified membranes, the adsorption efficiencies of 2,4-DCP and Bisphenol A were increased by 52.1% and 53.5%, respectively. The removal efficiencies of the modified fibrous membranes for Bisphenol A, 2,4-DCP and Triclosan reached above 90%, caused by the synergistic effect of enhanced membrane adsorption and laccase degradation. The more the phenolic pollutants were chlorinated, the harder the phenol was degraded by laccase.
81	Mixture of 2,4-D ⁽²⁾ (400 mg/L), 4-CP (5-45 mg/L), 2,4-DCP (20-45 mg/L) and 2,4,6-TCP (5-20 mg/L)	Inoculum: glucose-fed granules & plasmid pJP4 carrying donor strain <i>Pseudomonas putida</i> SM1443 Cultivation: 2,4-D (200 days) SBR ⁽³⁾ : - 3-4 h cycle time (50% vol. exch.) - Exposure time = 420 days - Temperature = 25 °C	The removal efficiency of 2,4-D was not affected by the addition of chlorophenols. The granules demonstrated a faster acclimation and higher degradation ability to 4-CP and 2,4-DCP than to 2,4,6-TCP. A dynamic change of the granule microbial community was observed.
82	4-CP (50-250 mg/L) in a mineral salt medium	Inoculum: <i>Arthrobacter chlorophenolicus</i> A6 Cultivation: 150 mg/L 4-CP, mineral salt medium and 0.1% yeast extract (48h, 28 °C, 180 rpm) Immobilization: Calcium alginate beads Packed Bed Reactor:	The toxic tolerance of <i>Arthrobacter chlorophenolicus</i> to 4-CP was improved by the entrapment in calcium alginate beads. The immobilized actinobacterium was able to degrade 4-CP at a high loading rate of 1275 mg/(L.d).

- Pyrex glass column: 912.5 mL
- Bed volume: 639 mL (86 g)
- HRT = 2-8 h
- pH = 7
- Temperature = 30 °C

⁽¹⁾ PCMX: 4-chloro-3,5-dimethylphenol

⁽²⁾ 2,4-D: 2,4-dichlorophenoxyacetic acid

⁽³⁾ SBR: Sequencing Batch Reactor

Table 3: The co-metabolic biodegradation of chlorophenolic pollutants by using pure isolated cultures

Reference	Target Pollutant(s)	Conditions	Highlights of the work
<i>Using suspended-growth biomass</i>			
30	Mixture of 4-CP (50-250 mg/L), PHENOL (50-200 mg/L) and SODIUM GLUTAMATE (200-1000 mg/L)	Inoculum: <i>Pseudomonas putida</i> ATCC 49451 Temperature = 30 °C	The biphasic growth pattern on the ternary substrate mixture was characterized by two exponential growth phases separated by an intermediate lag phase. Biphasic growth, involving inhibition among substrates, inactivation of cells and recoverability of the cells, occurs only at low phenol concentration, high 4-CP concentration and/or high SG concentration.
32	4-CP (15-40mg/L)	Inoculum: <i>Comamonas testosteroni</i> Experiments: <ul style="list-style-type: none"> - Co-metabolite: glucose (10-250 mg/L) or phenol (40-240 mg/L) - pH = 7.2 - Temperature = 30 °C 	4-CP transformation capacity was clearly deficient (only carbon and energy source). Phenol and glucose acted as primary growth substrate for 4-CP biodegradation. Complete 4-CP transformation: $S/X \leq 0.11$ when using phenol Simultaneous addition of phenol or glucose significantly reduces the minimum time required for complete 4-CP biodegradation.
54	2,4-DCP	Inoculum: <i>Phanerochaete chrysosporium</i> BKMF-1767 (CCTCC AF96007) Incubation: 3 days, 37 °C Cultivation: (i) 25-200 µM NaHS (4 h) and (ii) 20-50 mg/L 2,4-DCP (72 h) pH = 4.2-5.2	The H ₂ S donor NaHS successfully enhanced the degradation of 2,4-DCP by <i>P. chrysosporium</i> . H ₂ S can alleviate 2,4-DCP-induced oxidative stress and improve cell viability by increasing 2,4-DCP degradation.
61	PHENOL (200 mg/L)	Inoculum: <i>Pseudomonas sp.</i> cbp1-3 Experiments:	Phenol: degraded after 7 h with a lag phase of 5 h 4-CP: degraded after 40 h with a lag phase of 10 h

	4-CP (100 mg/L) Mixture of PHENOL (200 mg/L) and 4-CP (100 mg/L)	- pH = 7.2 - Temperature = 30 °C - Control = Succinate	Mixture: phenol & 4-CP degraded after 18 h & 22 h, respectively
74	2,4-DCP (25 - 400 mg/L)	Inoculum: <i>B. endophyticus</i> strain CP1R Cultivation: 50 mg/L 2,4-DCP and 1 g/L peptone (pH 7)	25 - 50 mg/L 2,4-DCP: complete degradation 100 - 400 mg/L 2,4-DCP: 75 - 80% degradation
75	4-CP (129 mg/L)	Inoculum: monooxygenase cloned and overexpressed from <i>Arthrobacter chlorophenolicus</i> A6 Experiments: - Co-metabolite: glucose (9 g/L)	A maximum removal efficiency of 82.7% was obtained.
78	Mixture of 4-CP (15 & 40 mg/L) and PHENOL (20–400 mg/L)	Inoculum: <i>Pseudomonas putida</i> LY1 Cultivation: phenol at pH 7.2 Experiments: - Variation of phenol and 4-CP concentration: o pH = 7.1–7.3 o Temperature = 25 °C o Shaker: 120 rpm - Variation of pH (3-9) & Temperature (5, 15, 25, 35 °C) o 4-CP: 15 mg/L o Phenol: 20-220 mg/L	4-CP as sole carbon source was not a suitable growth substrate for <i>P. putida</i> LY1. Temperature influenced both phenol and 4-CP removal rates significantly, and at 25 °C the highest degradation rate was observed. The pH ranges between 7 and 8 was found as optimal for cell growth and substrate degradation. Degradation and bacterial growth was rather poor in strong acidic conditions. The simultaneously biodegradation of both substrates was observed whereas the degradation of 4-CP increased only after the complete utilization of phenol. The removal time of phenol increased substantially in the presence of 4-CP (i.e., from 45 h to 115 h), combined with a long lag phase.
79	2-CP (130 mg/L) 3-CP (130 mg/L) 4-CP (130 mg/L)	Inoculum: <i>Stenotrophomonas maltophilia</i> KB2 Experiments: - Co-metabolite: phenol (280 mg/L) - After substrate depletion: subsequent identical dosage of phenol and chlorophenol - Temperature = 30 °C	Complete degradation of 4-CP was possible in the presence of phenol, whereas 2-CP and 3-CP degradation was incomplete. The co-metabolic degradation efficiency depended on the earlier exposure of bacteria to phenol and the position of the chlorine substituent in the aromatic ring. The time needed for biodegradation of recalcitrant pollutants can be reduced by the exposure of bacteria to easily degradable aromatic compounds.
83	4-CP (20 mg/L) 4- FLUOROPHENOL (20 mg/L)	Inoculum: <i>Raoultella planticola</i> WS2; <i>Pseudomonas</i> sp. OS2 Experiments:	The addition of saponins enhanced mostly the biodegradation of phenolic pollutants or reduced the time required to completely remove these impurities.

	4-BROMOPHENOL (20 mg/L)	<ul style="list-style-type: none"> - Co-metabolite: commercial product (extract from the bark of the <i>Quillaja Saponaria</i> Molina) and soap nut extract from the fruits of the <i>Sapindus Mukorossi</i> tree (both saponins) - Temperature = 30 °C - Exposure time = 25 days 	
Using attached-growth biomass			
72	PCP (500 mg/L) [and Cr ⁶⁺ (200 mg/L)]	<p>Inoculum: immobilized <i>Bacillus cereus</i> RMLAU1</p> <p>Cultivation: 500 mg/L PCP and 200 mg/L Cr⁶⁺ (60 h, pH 7, 35 °C)</p> <p>Experiments:</p> <ul style="list-style-type: none"> - Carbon sources: glucose, maltose and sucrose at 0.2, 0.4 and 0.6% (w/v) - Nitrogen sources: ammonium chloride, ammonium nitrate and urea at 0.1 & 0.2% (w/v) - Exposure time = 60 h - pH = 6.5-8.0 - Temperature = 25-40 °C 	<p>Bioreduction of Cr⁶⁺ to insoluble Cr³⁺ and simultaneous dechlorination of PCP was achieved by the isolate.</p> <p>The isolate is flexible under wide temperature and pH ranges (56% PCP and 74% Cr⁶⁺ remediated).</p> <p>Tetra-, tri-, di-chlorophenols and 6-chlorohydroxyguinol were detected as PCP dechlorination products.</p>
84	PHENOL (47 mg/L) 2-CP (64 mg/L) 4-CP (64 mg/L) 2,3-DCP (82 mg/L) 2,4,6-TCP (99 mg/L)	<p>Inoculum: <i>Rhodococcus rhodochrous</i> DSM6263</p> <p>Cultivation: 4 g/L yeast extract, 4 g/L glucose and 10 g/L malt extract (28 °C, pH 7.2)</p> <p>Immobilization:</p> <ul style="list-style-type: none"> - <u>Non-magnetically</u>: Agarose or <i>k</i>-carrageenan (2%) - <u>Magnetically</u>: Fe₃O₄ nanoparticles were added to the <i>k</i>-carrageenan-cell suspension. <p>Experiments:</p> <ul style="list-style-type: none"> - 14 g wet weight immobilized catalyst - Exposure time = 21-24 h - Temperature = 28 °C - pH = 7.2 	<p>The LC₅₀-value for <i>R. rhodochrous</i> DSM6263 (in suspension) with CPs were 129 mg/L, 193 mg/L and 82 mg/L for 2-CP, 4-CP and 2,3-DCP, respectively.</p> <p>Because the mass transfer resistance was reduced and the recovery of immobilized cells was facilitated, the economic competitiveness of this magnetic immobilization process was significantly enhanced compared with traditional immobilization.</p>
Using both suspended-growth and attached-growth biomass			
18	Mixture of	<p>Inoculum: <i>Pseudomonas putida</i> ATCC 49451</p> <p>Hollow fiber membrane bioreactor:</p>	Suspended cells:

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	4-CP (40-1000 mg/L) and PHENOL (200-1000 mg/L)	- 15 asymmetric polysulfone hollow fiber membranes - Suspended cells vs immobilized cells	- 600 mg/L phenol and 120 mg/L 4-CP: substrates were not degraded Immobilized cells: - 600 mg/L phenol and 120 mg/L 4-CP: substrates completely transformed - 1000 mg/L phenol and 1000 mg/L 4-CP: complete transformation within 45 h
77	2,4-DCP (10-800 mg/L)	Inoculum: <i>Bacillus insolitus</i> (isolated from a chemostate reactor – 800 mL/d influent containing 200 mg/L phenol and 10 mg/L 2,4-DCP) Cultivation: 200 mg/L phenol and 10 mg/L 2,4-DCP Immobilization: Alginate support Exposure time = 21 days	At low 2,4-DCP concentrations (10-50 mg/L), immobilized cells showed a higher removal of 2,4-DCP than suspended ones. At higher 2,4-DCP concentrations (50-200 mg/L), similar removal efficiencies were observed. Very high 2,4-DCP concentrations are inhibitive to the growth of either suspended or immobilized cells.

the bark of the *Quillaja saponaria* Molina and of the fruits of the *Sapindes mukorosi* tree were successfully used to enhance the biodegradation of 4-CP, fluorophenol and 4-bromophenol by *Raoultella planticola* or *Pseudomonas* sp.⁸³ Despite the results achieved by isolated bacteria, acclimated mixed cultures may provide additional benefits since different bacterial species in the mixed culture produce various enzymes required at each step of the transformation process. Table 4 and Table 5 represent relevant studies using mixed biomass cultures. Hereby, pre-acclimation of the biomass is important to enhance the rate and extent of biodegradation.^{32,68} Farrel and Quilty investigated the degradation of monochlorophenols by a mixed culture of *Pseudomonas* and *Actinomyces*, together with a species of white rot fungus *Trichoderma harzianum*, that was adapted for 238 h.¹⁵ A comparison between a non-acclimated and acclimated culture was performed by Sahinkaya and Dilek.⁶⁵ They concluded that acclimation resulted in an improved tolerance of microorganisms towards 2,4-DCP based on the disappearance of the lag phase and an improved COD removal efficiency at higher pollutant concentrations. A decrease in COD and 2,4-DCP removal efficiencies, and consequently an increase in effluent concentrations, accomplished by acclimated activated sludge was only observed by increasing the feed concentration above 150 mg/L 2,4 DCP.¹³ A COD removal efficiency of 94% was achieved during treatment of 2-CP-containing wastewater by pulp and paper mill secondary sludge acclimated to the chlorophenolic pollutant for 15 days in the presence of glucose.⁸⁵ In the context of acclimated biomass, an aerobic SBR offers a dynamic environment favorable for cell adaptation to toxic pollutants, resulting in superior performances for the treatment of PCP compared to continuous systems.⁸⁶ During the start-up procedure of the SBR, sodium acetate was used as growth substrate to trigger co-metabolic degradation of PCP. In a later phase, the concentration of the growth substrate was stepwise reduced, until the biomass was adapted in such a way it was able to grow on PCP as single substrate. Zhao et al. successfully acclimated activated sludge with 10 mg/L 4-CP and showed that by gradually elevating the 4-CP loads from 10 to 100 mg/L, the sludge performance was basically unaffected.⁸⁷ However, the sludge toxicity was increased. The ecological risk of the sludge toxicity towards land application using excess sludge was already highlighted by the same author.⁸⁸ The removal of pollutants in aqueous and sludge phases is not sufficient. The increased sludge toxicity was caused by the accumulation of dominant microbial secondary metabolites. Nitrifying sludge cultivated with p-cresol containing wastewater in a lab-scale CSTR was used for the treatment of a mixture containing 2-CP, phenol, p-cresol and p-OHB.⁷⁶ Compared to single 2-CP oxidation, the consumption rate of 2-CP was 2.4 times higher when 2-CP was present in a mixture of several phenolic pollutants, indicating the enhancement of the biodegradation process (both 2-CP oxidation and nitrifying process) by the metabolism. Furthermore, the co-metabolic biodegradation of 2-CP by acclimated biomass in the presence of phenol and resting cell conditions after depletion of phenol was observed by Aktas.¹⁹ Finally, Pérez-Alfaro et al. investigated the effect of

CP on the nitrifying process by comparing non-acclimated and acclimated (towards 4-MP) nitrifying biomass and concluded that acclimation was crucial for removing 2-CP without harming the nitrification process.¹⁶

The co-metabolic degradation of chlorophenols is usually performed by the addition of an extra carbon source. The effect of different carbon sources on the sludge performance and the microbial community for 4-CP wastewater treatment was discussed by Zhao et al.⁸⁹ The removal performance was not influenced, while significant differences in bacterial communities were observed. Instead of a carbon source, Moussavi et al. report the use of nitrate under anoxic conditions for improving the biological degradation of 2-CP by granular sludge acclimated to 100 mg/L 2-CP for 56 days.⁹⁰ They observed biodegradation efficiencies of 2-CP above 99% for inlet 2-CP concentrations up to 500 mg/L and demonstrated the coupling of the biodegradation of 2-CP to the use of nitrate. Chen et al. added NaHS to enhance the degradation of 2,4-DCP by *P. chrysosporium*.⁹¹ Co-metabolic biodegradation was not intended, however, H₂S or HS⁻, rather than other compounds derived from NaHS, can alleviate the 2,4-DCP-induced oxidative stress, and consequently improve cell viability by increasing 2,4-DCP degradation.

Finally, Wang et al. emphasized the need for kinetic models of co-metabolism since the inhibition form of both growth and non-growth substrates needs to be considered.⁷⁸ In this context, the 4-CP biodegradation kinetics by phenol-acclimated activated sludge was investigated by Lobo et al.⁹² Kinetic models contribute to obtain insights into the biodegradation interactions of the different substrates and the complex behaviors of different microorganisms in these circumstances. A SKIP-model (Sum Kinetics with Interaction Parameters) was applied in many studies.⁹³⁻⁹⁶ Also, alternative models are proposed to understand the mechanisms of the substrate interactions.⁹⁷⁻⁹⁹

3.3. Innovation in biological processes

To prevent substrate inhibition, a barrier is necessary to protect the microorganisms from the toxic pollutants, even when the substrate level in the bulk medium is inhibitory.¹⁸ In this context, the immobilization of microorganisms proved its feasibility to maintain cell growth and substrate degradation, mainly because of mass transfer limitations.¹⁰⁰ For example, alginate support utilized to immobilize *Bacillus insolitus* and *Arthrobacter chlorophenolicus* was investigated by Wang et al. and Sahoo and Panigrahy, respectively.^{77,82} Moreover, the successful degradation of 500 mg/L PCP was achieved by immobilized *Bacillus cereus*.⁷² Because of a synergistic effect of adsorption and degradation, Dai et al. obtained more than 90% removal of bisphenol A, 2,4-DCP and Triclosan by using Laccase-carrying electrospun fibrous membranes.⁸⁰ To enhance the economic competitiveness of the immobilized cultures, the recovery is an important aspect. Hence, Hou et al. used Fe₃O₄ nanoparticles to create a magnetic immobilized *Rhodococcus rhodochrous* to facilitate the recovery.⁸⁴ Lim et al. observed a consequently lower removal rate of 4-CP in an SBR compared to a moving bed SBR (MBSBR).⁷⁰ To tackle shock loadings of

Table 4: The single source biodegradation of chlorophenolic pollutants by using mixed cultures

Reference	Target Pollutant(s)	Conditions	Highlights of the work
<i>Using suspended-growth biomass</i>			
15	2-CP (200 mg/L) 3-CP (200 mg/L) 4-CP (200 mg/L)	Inoculum: mixed culture of <i>Pseudomonas</i> and <i>Actinomyces</i> , together with a species of white rot fungus, <i>Trichoderma harzanium</i> Experiments: - Exposure time = 238 h - pH = 7.0 - Temperature = 30 °C	<i>Meta</i> -cleavage of 2-CP and 3-CP resulted in a dead-end pathway leading to incomplete degradation, demonstrated the requirement for <i>ortho</i> -cleavage for successful degradation. Degradation via the <i>meta</i> -cleavage pathway led to complete degradation of 4-CP with a stoichiometric release of chloride.
86	PCP (10-20 mg/L)	Aerobic SBR: - 1 L glass vessel (0.6 L working volume) - Reactor exchange ratio: 0.5 (added volume/total volume) - SBR cycle: fill (5-20 min), reaction (1375-1390 min), settle (30 min), waste discharge (1 min) & draw (15 min) - DO = 3-4 mgO ₂ /L - Temperature = 27 °C Acclimation - First stage: - Batch mode - Acclimation time = 10 days - 100 mL inoculum and 100 mL feed solution (5 mg/L PCP & 50 mg/L sodium acetate) Acclimation - Second stage: - SBR mode - Acclimation time = 2 months - Increase of PCP concentration up to 35 mg/L - Reduction of sodium acetate concentration from 70 mg/L to 0 mg/L	At 10 mg/L PCP, the maximum specific removal rate was immediately achieved. The self-inhibitory effect increases when higher PCP concentrations were investigated. The aerobic SBR showed superior performance compared to continuous systems because of the dynamic environment favorable for cell adaption to toxic pollutants. The PCP degradation kinetics were accurately predicted by the Haldane equation (correlation coefficients > 0.99). The modified Haldane equation showed no significant improvements.
92	4-CP (80-460 mg/L)	Inoculum: activated sludge Cultivation: Lab-scale semi-continuous reactor enriched with 300 mg/L phenol (HRT = 3.3 days, SRT = 20 days, pH = 7)	The aerobic biodegradation of 4-CP by phenol-acclimated activated sludge was obtained without prior acclimation. The obtained respirometric profiles showed that the oxygenases is responsible for the first steps of the aerobic oxidation of 4-CP.

		Experiment:	The accumulation of an intermediate was observed since the COD removal was not complete at complete 4-CP degradation.
		- Volume = 500 mL	
		- Temperature = 30 °C	
101	4-CP (1000 – 2500 mg/L) + K ₂ Cr ₂ O ₇ (100 mg/L)	Inoculum: biomass previously acclimated to 4-CP Continuous two-phase partitioning bioreactor	The main attractiveness of the reactor configuration with polymeric tubing is the feature to biodegrade toxic organic molecules such as 4-CP without any contact between the microorganisms and the waste stream. The robustness of the reactor configuration was demonstrated over a wide range of loading and 4-CP removal was always higher than 98%.
		- 4L glass vessel	
		- 5.5 m Hytrel 8206 polymeric tubing	
		- Tubing/working volume = 4%	
		- HRT = 3-6 h	
		- VSS = 1 g/L	
		- DO = 3-4 mgO ₂ /L	
		- pH = 7.5	
		- Temperature = 27 °C	
102	4-CP (500 mg/L)	Inoculum: biomass previously acclimated to 4-CP Continuous two-phase partitioning bioreactor:	The tubing-based reactor was to remove 4-CP to residual concentrations in the range of 1-5 mg/L, even for high organic and hydraulic influent loads.
		- 5 L glass vessel	
		- 3.5 m Hytrel 8206 polymeric tubing	
		- Tubing/working volume = 2%	
		- VSS = 2 g/L	
		- Exposure time = 96 h	
		- Temperature = 25 °C	The so-called buffer effect of the polymer tubing allows the adaptation of the system to the increased organic load.
<i>Using attached-growth biomass</i>			
54	PCP (25 mg/L)	Inoculum: anaerobic granular sludge Cultivation: micro-aerobic granular sludge Reactor configuration:	During the acclimation stage, a progressive decrease of total CP and the residual PCP concentration and increase of MCP, DCP and TCP were observed.
		- USB ⁽¹⁾ reactor (HRT = 8 h, DO = 0.1-0.2 mgO ₂ /L, 30 °C)	TeCP was not observed throughout the experiment.
		- Exposure time = 39 days	The accumulation of TCP as major intermediate indicated that TCP degradation was the rate limiting step under low DO conditions (0.1-0.2 mgO ₂ /L).
			Reductive dechlorination was the predominant biotransformation process for PCP under the oxygen-limited condition.
<i>Using both suspended-growth and attached-growth biomass</i>			
48	2,4,6-TCP (10-260 mg/(L.days))	EGSB ⁽²⁾ reactor:	Bioaugmentation of EGSB clearly improved the chlorophenol removal efficiency and allowed also a moderate increase of the methane yield
		- Inoculum: anaerobic granular sludge from a lab-scale EGSB reactor enriched with 2,4-DCP	

- Cultivation: lab-scale EGSB reactor enriched with 2,4-DCP	although no significant effect was observed on COD reduction and overall dechlorination efficiency.
- Temperature = 30 °C	In the FBBR, both chlorophenol removal and overall dechlorination efficiencies were much higher than observed in the EGSB reactors (probably because of sorption onto GAC-biofilm).
FBBR ⁽³⁾ :	Mainly 4-CP, beside 2,4,6-TCP & 2,4-DCP, was detected in the effluent of both EGSB and FBBR.
- Inoculum: raw granular active carbon	In the aerobic SBR, 4-CP was completely converted within a range of HRT (16-80 h).
Bioaugmentation of EGSB and FBBR:	The rate of disappearance increased with the HRT because of the decrease of the maximum 4-CP concentration inside the SBR reactor after the filling stage.
- Strains: <i>Desulfitobacterium hafniense</i> PCP-1, <i>D. hafniense</i> TCP-A, and <i>D. chlororespirans</i>	
- Exposure time = 12 days	
Aerobic SBR:	
- Inoculum: activated sludge from a lab-scale SBR enriched with 4-CP (50 mg/(L.days)) over 2 months	
- HRT = 16-80 h, SRT = 30 days	

⁽¹⁾ USB: Upflow sludge fluidized bed reactor

⁽²⁾ EGSB: Expanded granular sludge bed

⁽³⁾ FBBR: Fluidized bed biofilm reactor

Table 5: Overview of recent studies focused on the co-metabolic biodegradation of chlorophenolic pollutants by using mixed cultures

Reference	Target Pollutant(s)	Conditions	Highlights of the work
<i>Using suspended-growth biomass</i>			
13	2,4-DCP (25-250 mg/L)	Inoculum: activated sludge Cultivation: 50 mg/L 2,4-DCP (several weeks) Activated sludge reactor: - Molasses-based wastewater: COD/N/P = 100/8/1.5 - HRT = 25 h - SRT = 10 days - pH = 7.5 - Temperature = 25 °C - DO = 2 mgO ₂ /L	COD and 2,4-DCP removal efficiencies decreased and effluent concentrations of COD and 2,4-DCP increased by increasing the feed contents above 150 mg/L 2,4-DCP. SVI-values also increases by increasing the feed concentration of 2,4-DCP.
16	2-CP (5-6 mg/L)	Inoculum: nitrifying biomass Cultivation: NH ₄ ⁺ and 4-MP ⁽¹⁾ (HRT = 2 days, pH 7.6, 25 °C)	The non-acclimated culture was not able to nitrify in the presence of 2-CP or consume the phenolic pollutant.

		Experiments: - pH = 7.5 - Temperature = 30 °C	The acclimated culture showed nitrifying activity after a lag phase of 12 h, whereas 2-CP consumption started around 75 h. Acclimation was crucial for removing 2-CP without losing nitrification.
19	2-CP (2-150 mg/L) + PHENOL (5-150 mg/L)	Inoculum: activated sludge Cultivation: 170 mg/L phenol and 110 mg/L 2-CP Temperature = 25 °C	Co-metabolic biodegradation of 2-CP by acclimated biomass was achieved in the presence of phenol (growth substrate) and at resting cell conditions even after phenol depletion. Optimum S_0/X_0 ratio for 4-CP: 1.5-5.5 mgCOD/mgMLSS (at S_0/X_0 ratio for phenol: 8.5 mgCOD/mgMLVSS)
26	2,4,6-TCP (100-450 mg/L)	Inoculum: aerobic activated sludge Granule cultivation: 5-10 mg/L 2,4,6-TCP + glucose or acetate (15 days) SBR: - HRT = 16, 24 and 48 h (50% vol. exch.) - SRT = 20 days - pH = 8-9 - Temperature = 30 °C - DO = 4-5 mgO ₂ /L	COD removal efficiency: 97% (TCP+glucose) and 95% (TCP+acetate) Biodegradation of 2,4,6-TCP occurred via formation of 3,5-dichlorocatechol pathway, followed by an <i>ortho</i> -cleavage. Intermediates: catechol, chlorocatechol
52	2-CP (20-140 mg/L)	Inoculum: pulp and paper mill secondary sludge (1.5 g MLVSS/L) Granule cultivation: 5-20 mg/L 2-CP (15 days) SBR: - Co-metabolite: glucose (1 g/L) - HRT = 8 h (50% vol. exch.) - SRT = 20 days	COD removal efficiency: 94% Biodegradation of 2-CP occurred via chlorocatechol pathway (modified <i>ortho</i> -cleavage). Biodegradation kinetics of 2-CP followed Haldane model for inhibitory substrate (maximum specific degradation rate at 100 mg/L 2-CP).
63	4-CP (211-1000 mg/L)	Inoculum: municipal activated sludge Experiments: - COD/N/P = 100/8/1.5 (molasses, urea and KH ₂ PO ₄) - MgSO ₄ (50 mg/L) - pH = 7.5 - Temperature = 25 °C - DO > 2 mgO ₂ /L	High 4-CP concentration adversely affected the RTBR ⁽²⁾ performance because of the toxic effects on the biofilm bacteria. High feed COD and high HRT were necessary for high 4-CP and toxicity removals at high feed 4-CP contents. Optimal conditions for 1000 mg/L 4-CP: 40 h HRT and feed COD = 6 gO ₂ /L; achieving 95% COD, 4-CP and toxicity removal
64	PCP (4.62-18.48)	Inoculum: anaerobic sludge (16 g/L) Experiments:	The degradation rate of PCP was enhanced by dosing sodium acetate at evenly distributed pulse and stepwise declining pulse modes.

		<ul style="list-style-type: none"> - Co-substrate: sodium acetate (0-5 g/L) - Dosing modes of co-substrate: one-step pulse, evenly distributed pulse and step-wise declining pulse - Zero valent iron = 1 g/L - VSS/TSS = 0.3 - Volume = 200 mL - Temperature = 35 °C 	The enhancement of the microbial activity was the main reason for the degradation improvement of PCP by dosing ZVI.
65	2,4-DCP (22-100 mg/L for non-acclimated and 76.6-194.5 mg/L for acclimated culture experiments)	<p>Inoculum: municipal activated sludge</p> <p>Cultivation: (semi-continuous reactor, SRT = 8 days)</p> <ul style="list-style-type: none"> - Non-acclimated culture: mixed cultures - Acclimated 2,4-DCP culture: stepwise increments up to 75 mg/L 2,4-DCP (5 months) - Acclimated 4-CP culture: 130 mg/L 4-CP <p>Co-metabolic experiments:</p> <ul style="list-style-type: none"> - Peptone - Temperature = 25 °C <p>Single carbon source experiments:</p> <ul style="list-style-type: none"> - Temperature = 25 °C 	<p>Acclimation improved the tolerance of microorganisms to 2,4-DCP:</p> <ul style="list-style-type: none"> - The lag phase observed for non-acclimated biomass disappeared. - The COD removal efficiency was not adversely affected, as observed by using non-acclimated biomass.
67	2,4-DCP (10 mg/L)	<p>UASB⁽³⁾ reactor:</p> <ul style="list-style-type: none"> - Co-substrate: glucose (3 g/L) - HRT = 2.07-10 h - Temperature = 35 °C 	By increasing the organic loading rate, the COD removal efficiency and methane gas percentage decreased from 84% to 33% and from 91% to 60%, respectively.
69	2,4-DCP (5-30 mg/L)	<p>Inoculum: municipal activated sludge</p> <p>Cultivated: 188 mg/L peptone and 563 mg/L sucrose (+ ethanol, 4.6% v/v, as electron donor for denitrification)</p> <p>SBR:</p> <ul style="list-style-type: none"> - HRT = 1.4 days - pH = 7.4-7.6 (2 g/L NaAc-sol.) 	<p>At 30 mg/L 2,4-DCP, a decrease of 2,4-DCP and nitrogen removal efficiencies of 41% and 25%, respectively, were observed.</p> <p>The nitrification process was completely inhibited, indicating the inhibitory effect of 2,4-DCP on the nitrification process.</p> <p>The inhibitory effect of 2,4-DCP was reversible when the addition of 2,4-DC ceased.</p>
71	2,6-DCP (20 mg/L)	Inoculum: municipal activated sludge	2,6-DCP had significant inhibitory effects on the COD removal and nitrification performance.

		<p>Cultivation: glucose-based synth. wastewater (pH 7; 25 °C; 30 days)</p> <p>Batch experiments:</p> <ul style="list-style-type: none"> - Exposure time = 9 h - pH = 7 - Temperature = 25 °C - DO > 5 mgO₂/L <p>Long-term SBR experiments:</p> <ul style="list-style-type: none"> - SRT = 14 days - pH = 7 - Temperature = 25 °C - DO > 5 mgO₂/L 	2,6-DCP favored a reduction in the sludge growth (around 40%).
73	2,4,6-TCP (10-500 mg/L)	<p>Inoculum: municipal activated sludge</p> <p>Cultivation: 10 mg/L TCP + 260 mg/L glucose + 415 mg/L NaAc (21 days)</p> <p>SBAR⁽⁴⁾:</p> <ul style="list-style-type: none"> - HRT = 12 h (50% vol. exch.) - pH = 7.0-7.8 - Temperature = 20 °C 	<p>2,4,6-TCP and COD removal efficiencies were above 93% and 90%, respectively.</p> <p>The specific degradation rate peaked at 61 mg/(gMLVSS.h) at the 2,4,6-TCP concentration of 400 mg/L.</p>
76	Mixture of 2-CP, PHENOL, <i>p</i> -CRESOL and <i>p</i> -OHB ⁽⁵⁾ (50 mgC/L of each pollutant)	<p>Inoculum: nitrifying sludge (lab-scale CSTR)</p> <p>Cultivation: 1.0 y; 25 °C; HRT = 1.8 days; <i>p</i>-cresol Loading rate = 89 mg/(L.days)</p> <p>Experiments:</p> <ul style="list-style-type: none"> - pH = 7.0 - DO = 4.0 mgO₂/L 	<p>Mixture of phenolic pollutants had a positive effect on both 2-CP oxidation and nitrifying process.</p> <p>Kinetic order: <i>p</i>-cresol > <i>p</i>-OHB > 2-CP > NH₄⁺ > phenol</p> <p>Compared to single 2-CP oxidation, the consumption rate of 2-CP was 2.4 times higher when 2-CP was present in the mixture of several phenolic pollutants.</p>
87	4-CP (10-100 mg/L)	<p>Inoculum: municipal activated sludge</p> <p>SBR:</p> <ul style="list-style-type: none"> - Volume = 5 L - 8 h cycle with 7 h operation period - Volumetric exchange ratio = 70% - HRT = 8 h - MLSS = 2500 mg/L <p>Experiments:</p> <ul style="list-style-type: none"> - Co-substrate: methanol (COD = 300 mg/L) - DO = 1.5 mgO₂/L 	<p>After the successful acclimation of the sludge with 10 mg/L 4-CP, the sludge performance was not affected with the gradually elevated influent 4-CP loads from 10 mg/L to 100 mg/L.</p> <p>The elevated influent 4-CP loads caused a gradually increase of the sludge toxicity.</p>

		<ul style="list-style-type: none"> - Exposure time = 145 days - pH = 7.4 - Temperature = 25 °C 	
88	4-CP (10 mg/L)	<p>Inoculum: municipal activated sludge</p> <p>SBR:</p> <ul style="list-style-type: none"> - Volume = 5 L - HRT = 12 h - SRT = 20 d - MLSS = 2500 mg/L <p>Experiments:</p> <ul style="list-style-type: none"> - Co-substrate: methanol (COD = 300 mg/L) - DO = 1.5 mgO₂/L - Exposure time = 70 days - pH = 7.4 - Temperature = 25 °C 	<p>The toxicity of the 4-CP acclimated sludge significantly exceeded the blank sludge, although 4-CP was not detected in the effluent.</p> <p>The microbial diversity led to the accumulation of the dominant microbial secondary metabolites and in turn resulted in more extent of toxicity in the 4-CP acclimated sludge.</p> <p>The biodegradation process of 4-CP was coupled with the release of chloride ion in the solution.</p>
89	4-CP (1.5 mg/L) in artificial wastewater	<p>Inoculum: municipal activated sludge</p> <p>SBR:</p> <ul style="list-style-type: none"> - Volume = 5 L - 12 h cycle with 11 h operation period - Volumetric exchange ratio = 70% - MLSS = 2.5 g/L - SVI = 105 mL/gMLSS <p>Experiments:</p> <ul style="list-style-type: none"> - Co-substrate: dissolved starch; sodium acetate (both in the COD range of 320-420 mg/L) - DO = 1.5 mgO₂/L - Exposure time = 50 days - pH = 7.2 - Temperature = 19 °C 	<p>During the first 6 days, the 4-CP concentration of the effluent exceeded 0.6 mg/L, indicating the inhibition of the sludge activity caused by the toxicity of 4-CP. In the following period, the 4-CP concentration in the effluent decreased below 0.2 mg/L by the enrichment of 4-CP degrading bacteria.</p> <p>The removal performance of 4-Cp was not influenced by different carbon sources.</p> <p>The dominant genus was <i>Gemmobacter</i>, independent of the carbon source.</p>
Using attached-growth biomass			
49	Mixture of PCP (1.5 mg/L), HCB (0.1 mg/L) ⁽⁶⁾ , PCB (0.1 mg/L) ⁽⁷⁾ ,	<p>Inoculum: culture XX01 enriched from Xiangxi River sediment</p> <p>Cultivation: mineral salt medium (50 mg NaAc)</p> <p>Experiment:</p>	<p>Dechlorination of HCB, PCP, PCB, HCH and PCE was achieved.</p> <p>Intermediates: 1,3-DCB⁽¹⁰⁾; 1,4-DCB; 3,4-DCP; 3,5-DCP & PCB</p>

	HCH (1.0 mg/L) ⁽⁸⁾ and PCE (7.5 mg/L) ⁽⁹⁾	<ul style="list-style-type: none"> - Reactor volume = 2 L (glass bottles capped with Teflon-coated septa) - 20 mL inoculum - Temperature = 22-24 °C 	
66	2-CP (30 mg/L) 2,4-DCP (30 mg/L)	Sequential UASB & RBC ⁽¹¹⁾ <ul style="list-style-type: none"> - Flow rate = 1041 mL/h - UASB <ul style="list-style-type: none"> o Volume = 12.5 L o HRT = 12 h o VSS = 19.0-22.0 kg VSS/m³ (UASB-I); 16.0-19.0 kg VSS/m³ (UASB-II) o SS = 48.0-49.0 kg SS/m³ (UASB-I); 35.0-36.5 kg SS/m³ (UASB-II) - RBC <ul style="list-style-type: none"> o Volume = 24 L (RBC-I for 2-CP degradation) 30 L (RBC-II for 2,4-DCP degradation) o VSS biofilm = 6.2 mg/cm² (RBC-I); 7.5 mg/cm² (RBC-II) o VSS/SS ratio = 0.80 Experiment: <ul style="list-style-type: none"> - Carbon sources: sodium formate (5.0 g/L); sodium propionate (1.4 g/L); glucose (1.4 g/L); methanol (1.25 mL/L) - Supplementary feed for RBC: <ul style="list-style-type: none"> o Carbon source: sodium formate (11.3 g/L); sodium propionate (3.2 g/L); glucose (3.2 g/L); methanol (2.8 mL/L) o Flow rate = 85 mL/h - Exposure time = 25-30 days - Temperature = 27 °C 	By using of glucose and methanol, higher chlorophenol removal efficiencies in the UASB were achieved compared to formate or propionate. The effect of shock loadings was most visible in the UASB reactor. The chlorophenol and COD removal as well as the gas production decreased slightly. The sequential reactors withstood the shock loadings, resulting in the (almost) complete removal of 2-CP and 2,4-DCP.
90	2-CP (50-700 mg/L)	Inoculum: municipal activated sludge Cultivation: 100 mg/L 2-CP (56 days)	The AnBR tolerated inlet 2-CP concentrations of up to 500 mg/L (2-CP biodegradation efficiency > 99% at HRT = 4h).

		Granular AnBR ⁽¹²⁾ : <ul style="list-style-type: none"> - Mass ratio of NO₃⁻ to 2-CP = 5 - HRT = 2-24 h - Salinity = 0.5-40 g/L NaCl - Temperature = 24 °C - Exposure time = 167 days 	The increase of inlet 2-CP concentration had a more inhibitory effect on the 2-CP biodegradation than the increase of the inlet flowrate. The AnBR efficiently recovered following different shock loading conditions. The biodegradation of 2-CP was coupled to the use of nitrate.
103	4-CP (1-15 mg/L)	Inoculum: aerobic granular sludge Cultivation: high-strength nitrogen wastewater containing glucose (SBR with HRT = 12 h & 50% vol. exch.) Batch exposure experiments: <ul style="list-style-type: none"> - Synthetic wastewater + 4-CP - Exposure time = 6 h - DO > 2 mgO₂/L - Temperature = 25 °C 	At 4-CP concentrations of 5 and 10 mg/L, NH ₄ -N removal efficiencies decreased to 87.2% and 41.2%, respectively (control: 99.8%). Heterotrophic and autotrophic bacteria activity were significantly inhibited by the presence of 4-CP. The presence of 4-CP also played an important role on the EPS production.
104	Mixtures of <i>o</i> -CRESOL (40-100 mgC/L), <i>o</i> -CP (20-30 mgC/L) and <i>p</i> -NP ⁽¹³⁾ (45-200 mgC/L)	Inoculum: activated sludge Cultivation: <i>p</i> -NP, granular Batch experiments: <ul style="list-style-type: none"> - pH = 7.5 - Temperature = 25 °C - DO = 4 mgO₂/L Long-term SBR experiments: <ul style="list-style-type: none"> - HRT = 1-2 days (25% vol. exch.) - pH = 7-8 - Temperature = 20-25 °C 	The influent containing <i>p</i> -NP, <i>o</i> -cresol and <i>o</i> -CP caused the complete failure of the SBR. The phenolic-degrading species in the granules were identified as <i>Pseudomonas</i> sp., <i>Acinetobacter</i> sp. and <i>Arthrobacter</i> sp.
Using both suspended-growth and attached-growth biomass			
68	4-CP (50 mg/L)	Inoculum: municipal activated sludge Experiments: <ul style="list-style-type: none"> - 4-CP/NaAc = 0.05 (GSBR⁽¹⁴⁾); 0.05-0.13 (SBR); 0.06 (MBR) - pH = 7.0 - Temperature = 20 °C 	GSBR: complete 4-CP removal; 4.65 mg _{4-CP} /(gMLVSS.h) SBR: complete 4-CP removal; 6.91 mg _{4-CP} /(gMLVSS.h) MBR: complete 4-CP removal; 3.76 mg _{4-CP} /(gMLVSS.h) GSBR & SBR were able to withstand a 4-CP volume organic loading rate two folds higher than the MBR.
70	4-CP (200-600 mg/L)	Inoculum: municipal activated sludge Cultivation: feed solution based on peptone, sucrose and NaAc (SRT = 40 days) SBR vs MBSBR ⁽¹⁵⁾ :	The removal rate of 4-CP in the SBR was systematically lower than in the MBSBR. At high initial concentration of 4-CP (600 mg/L), the removal rate of 4-CP decreased significantly compared to lower initial concentrations.

-
- 8-40% (v/v) packing
 - pH = 7.5
-

- ⁽¹⁾ 4-MP: 4-methylphenol
- ⁽²⁾ RTBR: Rotating perforated tube biofilm reactor
- ⁽³⁾ UASB-reactor: Upflow anaerobic sludge blanket reactor
- ⁽⁴⁾ SBAR: Sequencing batch airlift reactor
- ⁽⁵⁾ *p*-OHB: *Para*-hydroxybenzaldehyde
- ⁽⁶⁾ HCB: Hexachlorobenzene
- ⁽⁷⁾ PCB: Polychlorinated biphenyl
- ⁽⁸⁾ HCH: Hexachlorocyclohexanes

- ⁽⁹⁾ PCE: Tetrachloroethane
- ⁽¹⁰⁾ DCB: Dichlorobenzene
- ⁽¹¹⁾ RBC: Rotating Biological Contactor
- ⁽¹²⁾ AnBR: Anoxic baffled reactor
- ⁽¹³⁾ *p*-NP: *Para*-nitrophenol
- ⁽¹⁴⁾ GSB: Granular sequencing batch reactor
- ⁽¹⁵⁾ MBSBR: Moving bed sequencing batch reactor

chlorophenolic toxic pollutants, Majumder and Gupta used a sequential UASB-RBC concept.⁶⁶ Obviously, the UASB encountered the most effects of the shock loading, resulting in a slight decrease of chlorophenol degradation and gas production. However, the sequential reactors were able to intercept the higher 2-CP and 2,4-DCP concentrations. Angelucci *et al.* and Tomei *et al.* used a continuous two-phase partitioning bioreactor to enhance biodegradation of 4-CP and xenobiotic removal.¹⁰¹⁻¹⁰² The direct contact between the toxic wastewater and the microorganisms is prevented by this configuration. Furthermore, the cells are exposed to sub-inhibitory concentration levels since the metabolic process regulates the gradual substrate delivery.

Furthermore, Puyol *et al.* used granular active carbon (GAC) on which biofilm can grow and applied the GAC-biofilm in a fluidized bed bioreactor (FBBR) for the removal of 2,4,6-TCP.⁶⁰ The performances of the FBBR were compared to the results achieved by an expanded granular sludge bed (EGSB) reactor. Anaerobic conditions were applied in both reactors. In the FBBR, both chlorophenol removal and overall dechlorination efficiencies were much higher than observed in the EGSB reactor, probably because of sorption onto the GAC-biofilm. Mainly 4-CP was detected in the effluent of both EGSB reactor and FBBR. Further aerobic treatment in an SBR was performed at different hydraulic retention times (HRT). The complete transformation of 4-CP was observed within the applied range of HRT (16-80 h), and the rate of disappearance increased with the HRT. Despite the inferior results of the anaerobic granular sludge observed by Puyol *et al.*, aerobic granular sludge has recently attracted wide attention because of the higher biomass concentrations, a denser and stronger microbial aggregate structure, and excellent settling capacity compared to activated sludge.^{48,103} The dissolved oxygen gradient throughout the granule results in different zones in which a wide range of micro-environments are detected, i.e., heterotrophic aerobic bacteria grow on the outside, facultative anaerobic bacteria in the core and ammonium-oxidizing bacteria in the intermediate layer of the granules.¹⁰⁵ The granule cultivation of aerobic activated sludge is reported by Khan *et al.*^{26,52} Both studies observed COD removal efficiencies above 94%. Fernandez *et al.* cultivated granular sludge acclimated to *para*-nitrophenol (p-NP) for the removal of a mixture of *o*-cresol, *o*-CP and *p*-NP.¹⁰⁴ The phenolic degrading species in the granules were identified as *Pseudomonas sp.*, *Acinetobacter sp.* and *Arthrobacter sp.* Furthermore, the degradation of 50 mg/L 4-CP in three different reactors (SBR, granular SBR (GSBR) and MBR) was compared by Carucci *et al.*⁶⁸ The GSBR and SBR were able to withstand a 4-CP volume organic loading rate twice as high as that of the MBR. Finally, Ma *et al.* demonstrated a faster acclimation and higher degradation ability of granules to 4-CP and 2,4-DCP compared to 2,4,6-TCP.⁸¹

4. Ozonation of chlorophenolic pollutants

Ozone as a single oxidant has showed a satisfactory effectiveness for the degradation of a wide variety of organic pollutants, including chlorophenols.¹⁰⁶ Moreover, many studies report efficiency improvements by combining ozone with catalysts, UV-light and/or other oxidants. Therefore, Table 6 and Table 7 include the highlights of CP degradation and biodegradability improvement, respectively, in single substrate solutions. Studies regarding CP degradation and biodegradability improvement in multiple substrate solutions are summarized in Table 8 and Table 9, respectively.

4.1. Degradation pathways and intermediate products

Ozone oxidizes the target molecules either directly or through the formation of hydroxyl radicals. The predominant mechanism depends on the pH of the solution and the target molecules. The direct pathway ($\text{pH} \approx 2$) is based on the reaction of ozone with pollutants with specific functional groups through selective reactions such as electrophilic, nucleophilic or dipolar addition reactions.¹⁰⁷ Under acidic conditions, rather low kinetic constants in the range of 1-1000 L/(mol.s) were observed.³⁹ At high pH (> 9), the ozone process favors the production of hydroxyl radicals by which the system becomes more effective in oxidizing complex mixtures. The increase in ozone degradation rates by increasing pH is confirmed by several studies.¹⁰⁸⁻¹¹² Based on their standard oxidation potentials, hydroxyl radicals are stronger oxidants than molecular ozone.¹¹³ In the system O_3/OH^- , the hydroxide ion reacts with ozone to yield superoxide anion radicals, which in turn are involved in a series of reactions that yield hydroxyl radicals. In the range of pH 4 - 9, both reaction pathways are active.³⁹

Pera-Titus *et al.* proposed a general reaction scheme for the ozonation of chlorophenolic substances in aqueous solutions (Figure 3).³⁹ Several authors achieved satisfactory results at high pH values.^{22,114-116} The ozone decomposition proceeds through a chain reaction in the formation of highly reactive hydroxyl radicals.³⁹ However, direct ozonation can also be important, even at elevated pH values. Indeed, when the concentration of fast reacting species with molecular ozone is sufficiently high, direct ozonation will be the dominating mechanism, even at higher pH values. Within this context, Hautaniemi *et al.* observed no contribution of hydroxyl radical reactions to the chlorophenol oxidation rate under alkaline conditions, probably because of the high reaction rate of phenolate ions with ozone.²¹ Moreover, Hong and Zeng concluded that the degradation of PCP was initiated via direct nucleophilic attack by ozone as predominant pathway, whereas both ozone and secondary hydroxyl radicals were likely to be involved in subsequent degradation of intermediates.²³

Also, Benitez *et al.* observed an increase in the degradation rate with increasing pH (from pH 2 to 9) and ascribed this to (i) the dissociation of phenols to phenolate ions, which are able to react with ozone faster than the non-dissociated species and (ii) the faster production of hydroxyl radicals at

Table 6: Overview of recent studies focused on the degradation of chlorophenolic pollutants present in single substrate solutions

Reference	Target Pollutant	Conditions of ozone-based oxidation technique	Highlights of the work
<i>Degradation by ozonation</i>			
21	2-CP (52.7 mg/L) 4-CP (52.7 mg/L) 2,4-CP (66.8 mg/L)	<u>Ozonation</u> - Ozone concentration in gas phase = 0.5 mg/L - pH = 9.5	The chlorophenol ozonation reactions in alkaline solutions followed pseudo-first order kinetics. In the final phase, the simulations predicted faster chlorophenol decomposition and lower ozone consumption than observed experimentally because of the formation of intermediate products rapidly reacting with ozone. Hydroxyl radical reactions did not make any contribution to the chlorophenol oxidation rate under alkaline conditions, probably because of the high reaction rate of the phenolate ions with ozone at pH conditions favorable for hydroxyl radical oxidation.
114	2-CP (50 mg/L) 4-CP (50 mg/L) 2,4-DCP (65 mg/L) 2,4,6-TCP (80 mg/L) 2,3,4,6-TeCP (90 mg/L) PCP (105 mg/L)	<u>Ozonation</u> - Reactor: bubble column - Ozone concentration in gas phase = 0.5 mg/L - pH = 2.5 or 9.5 - Reaction time = 2.5-45 min - Volume = 100 mL	Ozonation under alkaline conditions led to the most efficient removal of all of the CPs studied. The removal of CPs in alkaline conditions was accompanied by a decrease of pH revealing the presence of acidic oxidation products, also the color of the reaction mixture changes indicates the formation of new chromophores (most probably quinones and their derivatives); towards the end of the overall treatment time, the color of the mixture disappeared. Typical intermediate products formed during oxidation of chlorophenols were chlorobenzenediols. Oxidation of chlorophenols was effective, but incomplete dechlorination indicated the presence of chlorinated intermediates (chlorinated aliphatic structures).
117	PCP (1.25-2.5 mg/L)	<u>Ozonation</u> - Ozone concentration in liquid phase = 0.72-13.44 mg/L - pH = 2-7 - Reaction time = 5-20 min - Temperature = 10-40 °C	The overall reaction is second order with first order each for the concentrations of PCP and ozone. Depending on the solution pH, PCP can be present in both molecular and ionic forms, and pentachlorophenolate reacts much faster with ozone than PCP.
118	PCP (2.85-10 mg/L)	<u>Ozonation</u> - pH = 2.5-9 - Temperature = 20 °C - Volume = 500 mL	The stoichiometric ratio for the oxidation of PCP by ozone was 1.5 mol of ozone consumed per mol of PCP reacted. Rate constants between 0.67×10^5 L/(mol.s) (at pH 2.5) and 3.15×10^7 L/(mol.s) (at pH 9) were determined.

			<p>The specific ozonation rate constant for the dissociated and undissociated form of PCP were determined, i.e., 9.10×10^6 L/(mol.s) and 1.00×10^4 L/(mol.s), respectively.</p> <p>Main intermediate products: tetrachlorocatechol, tetrachlorohydroquinone and tetrachloro-<i>p</i>-benzoquinone</p>
119	PCP (10-80 mg/L)	<p><u>Ozonation</u></p> <ul style="list-style-type: none"> - Reactor: microchannel - Superficial gas velocity = 0.1-0.5 m/s - Superficial liquid velocity = 0.5-10.5 m/s - Ozone concentration in gas phase = 13-40 mg/L - pH = 6-12 	<p>Typical Taylor flow patterns were observed.</p> <p>The microchannel reactor demonstrated an excellent performance in the ozone mass transfer efficiency and the ozonation of PCP.</p> <p>The ozonation of PCP was a pseudo-first order reaction, and the apparent reaction rate constant, observed at different PCP concentrations (10-80 mg/L), varied between 2.10-2.13 1/s.</p>
120	4-CP (6 mg/L) 2,4-DCP (6 mg/L) PHENOL (6 mg/L)	<p><u>Ozonation</u></p> <ul style="list-style-type: none"> - Ozone concentration in liquid phase = 7-40 mg/L - Ozone dosage = 2.6 mgO₃/mgC, 2.0 mgO₃/mgC and 2.6 mgO₃/mgC for phenol, 4-CP and 2,4-DCP, respectively - Reaction time = 96 h - Volume = 5 L 	<p>Removal efficiencies for phenol, 4-CP and 2,4-DCP were determined to be 98.9%, 99.8% and 98.9%, respectively.</p>
121	PHENOL	<p><u>Ozonation</u></p> <ol style="list-style-type: none"> 1. Pilot with ejector <ul style="list-style-type: none"> - Reactor: tank with circulation containing heat exchanger, ejector and mixture tube (0.5-1 m³/h) - O₂/O₃ gas flow rate = 0.1-0.8 m³/h - Ozone concentration in gas phase = 5% in weight - pH = 2.7-11.3 - Temperature = 20-35 °C - Volume = 30 L 2. Bubble column <ul style="list-style-type: none"> - Reactor: cylindrical body with spherical head, with circulation (0.16 m³/h) - O₂/O₃ gas flow rate = 0.2 m³/h - Ozone concentration in gas phase = 5% in weight - pH = 2.7-11.3 	<p>The ejector had higher volumetric mass transfer coefficient and gas retention rate.</p> <p>By increasing the pH, the ejector achieved better results for phenol degradation.</p> <p>For diffusion-controlled regime (ozonation at high pH), the ejector allowed treatment of important flow rate in a short time, with an efficient mass transfer.</p>

		-	Volume = 2.5 L	
Degradation by ozone-based processes				
22	4-CP (40 mg/L) 2,4-DCP (50 mg/L) 2,4,6-TCP (60 mg/L) 2,3,4,6-TeCP (70 mg/L)	<u>Ozonation and O₃/UV</u>	<ul style="list-style-type: none"> - UV light: high pressure mercury vapor lamp (185-436 nm) - pH = 2 or 9 - Temperature = 25 °C - Volume = 350 mL 	<u>Ozonation</u> <p>Ozone is more reactive with higher substituted CPs whereas hydroxyl radicals react faster with those chlorophenols having lower number of chlorine atoms.</p> <p>For ozonation in acidic conditions, the increase in the number of chlorine atoms in the aromatic ring provided an increase in the degradation rate (the presence of more chlorine atoms enhanced the dechlorination step). The most important fact observed is the increase of the rate constant (pseudo-first order) with increasing pH (from 2 to 9) because of the faster production of hydroxyl radicals at alkaline pH and the dissociation of phenols to phenolate ions that are able to react faster with ozone than the non-dissociated species.</p> <p><u>O₃/UV</u> This combination accelerates the decomposition rate, with an extremely high rate constant for 4-CP, and lower rates for the other CPs. All CPs, apart from 4-CP, presented slightly increasing rate constants when the substituent chlorine atoms increase.</p>
122	2,4-D (300 mg/L)	<u>O₃/UV & photocatalytic ozonation:</u>	<ul style="list-style-type: none"> - Photocatalyst: titanium dioxide coated glass fiber - UV-light: black light (6 x 20 W) - Ozone concentration in gas phase = 1.4 mg/L - O₂/O₃ gas flow rate = 310 mL/min - pH = 3-11 - Temperature = room temperature (18-22 °C) - Volume = 500 mL 	<u>O₃/UV</u> <p>By increasing both the ozone concentration in the gas phase and the pH, the degradation rate of 2,4-D increased.</p> <p>At pH 9, a first-order rate constant of 0.00544 1/min was observed.</p> <p><u>Photocatalytic ozonation</u> At pH 9, a first-order rate constant of 0.00166 1/min was observed. The lower rate constant was described to either the inhibiting effect of O₃ on photocatalysis or the decay of ozone at the large catalyst surface without any reaction with 2,4-D. Compared to O₃/UV, higher mineralization rate of TOC was observed (from 139 mg/L to 27.4 mg/L, instead of 118 mg/L).</p>
123	4-CP (200 mg/L) 4-NP ⁽¹⁾ (200 mg/L) PHENOL (200 mg/L)	<u>Ozonation, O₃/UV, catalytic ozonation and photocatalytic ozonation</u>	<ul style="list-style-type: none"> - Reactor: tubular photoreactor - Catalyst: TiO₂ (1.5 g/L) - UV light: high pressure mercury lamp (700 W, 238-579 nm) 	<p>The highest mineralization rate of the phenolic pollutants was achieved by photocatalytic ozonation (100%, 90% and 75% mineralization for phenol, 4-CP and 4-NP, respectively).</p> <p>Identified intermediates 4-CP: hydroquinone, resorcinol, benzoquinone, oxalic acid, maleic acid, fumaric acid, glyoxylic acid, formic acid</p> <p>All processes followed pseudo-first order kinetics for phenol removal.</p>

		<ul style="list-style-type: none"> - O₂/O₃ gas flow rate = 51 L/h - Ozone concentration in gas phase = 5 mg/L - pH = 2.9-6.1 - Volume = 900 mL 	
124	2,4-D ⁽²⁾ (10 mg/L)	<u>Ozonation, O₃/UV and photocatalytic ozonation</u> <ul style="list-style-type: none"> - Catalyst: TiO₂ silica gel powder (5 g/L) - UV light: UV tube lamp (10 W, 254 nm) - Air/O₃ gas flow rate = 1.0 L/min - Ozone dosage in gas phase = 0.5-3 mg/(L.min) - pH < 4.6 - Reaction time = 80 min - Temperature = 10-30 °C - Volume = 1.3 L 	<p>Decay of 2,4-D and TOC by all oxidation processes was accurately described by the first-order reaction kinetic.</p> <p>Removal of 2,4-D and TOC was significantly enhanced in the photocatalytic ozonation system, and further enhancement was observed with larger ozone supplies (2,4-D k-value of photocatalytic ozonation was 1.4-1.7 times higher than the sum of corresponding k-values of ozonation and UV/TiO₂).</p>
125	2-CP (100 mg/L)	<u>Catalytic ozonation</u> <ul style="list-style-type: none"> - Catalyst: Ni-containing fly ash and sawdust - Liquid/solid ratio = 2:1, 5:1, 10:1 - pH = 7-11 - Reaction time = 12 min - Temperature = 20 °C - Volume = 200 mL 	<p>The increase of the catalyst ratio seemed to increase the catalytic activity sites, which could improve the 2-chlorophenol ozonation oxidation reaction rate as well.</p> <p>The increase of pH resulted in the decrease of 2-chlorophenol removal efficiency with the catalyst.</p> <p>The 2-CP ozonation process followed the pseudo-first order kinetic model, and the reaction rate constant of catalytic ozonation increased by 267%, compared to single ozonation.</p> <p>Oxidation products: first phenol, then oxalic acid as main end product</p>
126	2,4-D (440 mg/L)	<u>Ozonation, O₃/UV and photocatalytic ozonation</u> <ul style="list-style-type: none"> - Catalyst: TiO₂ (Degussa P-25; 2g/L) and Fe(II) (10-85 mg/L) - UV light: 6 W Philips black-light fluorescent lamp (300-420 nm) - Ozone dosage = 1.4 g/h - pH = 2-10 - Reaction time = 120 min - Temperature = 25 °C - Volume = 100 mL 	<p>Both catalysts improved the reaction efficiency when compared with only ozone and UV light, and best results were obtained with Fe(II)/UV/O₃ combination.</p> <p>Dechlorination seemed to be one of the first steps of the degradation reaction.</p>
127	2-CP (65 mg/L) 2,4-D (22 mg/L) BDDAB ⁽³⁾ (38 mg/L) BNS ⁽⁴⁾ (27 mg/L)	<u>Ozonation, O₃/UV, photocatalytic ozonation</u> <ul style="list-style-type: none"> - Reactor: ozone bubbling tube followed by solar photoreactor - Catalyst: TiO₂ (nanoparticulate; 0.5-2.0 g/L) 	<p>The mineralization process was enhanced by the combination of photocatalysis and ozonation methodologies.</p> <p>The dechlorination of 2-CP occurred more rapidly by increasing the amount of TiO₂ catalyst, with the optimal loading being 1.0 g/L.</p>

	BPA ⁽⁵⁾ (23 mg/L) DBS ⁽⁶⁾ (35 mg/L)	<ul style="list-style-type: none"> - UV light: solar light harvest (area = 3.06 m²) - Ozone concentration in liquid phase = 3 mg/L - Temperature = increased to ± 45 °C - Volume = 100-500 L (from the feed tank continuously circulated over bubbling tube and photoreactor at a rate of 7 L/min) 	<p>Both the aromatic ring cleavage and dechlorination were presumed to occur simultaneously in the first initial step of the photocatalytic ozonation of 2-CP.</p> <p>The complete mineralization of 2,4-D, BPA, DBS and BNS present in the simulated wastewaters was achieved within 3 h of photocatalytic ozonation, whereas BDDAB was somewhat recalcitrant to photodegradation.</p>
128	PHENOL (93-105 mg/L)	<p><u>Ozonation, O₃/UV, O₃/H₂O₂ and O₃/UV/H₂O₂</u></p> <ul style="list-style-type: none"> - Reactor: bubble column with a co-current circulation of gas and liquid - UV light: mercury vapor lamp (253.7 nm) - O₂ gas flow rate = 100 L/h - ozone dosage = 0.2-0.3 g/h - pH = 3-9 - Temperature = 16-20 °C - Volume = 2.5 L 	<p>None of the ozone combinations (O₃/UV, O₃/H₂O₂ and O₃/UV/H₂O₂) improved the degradation rate (pseudo-first order kinetic) of the ozone process (alkaline conditions), even resulting in a limited inhibitory effect. Based on the obtained degradation rate and the lower costs, the most appealing choice for phenol degradation was ozonation (even above other oxidation techniques such as UV/H₂O₂ and Fenton).</p>
129	2,4,6-TCP (50 mg/L)	<p><u>Adsorption - ozonation</u></p> <ul style="list-style-type: none"> - Adsorbent: high-silica FAU⁽⁷⁾ zeolite - Adsorption: 400 mL of 50 mg/L + 10 g zeolite; 2 h; room temperature; pH 6.2-6.5 - Ozone concentration in gas phase = 45-50 mg/L - Ozone concentration in water = 7 mg/L - After ozonation, the adsorption was repeated - Temperature = room temperature 	<p>TCP adsorption onto adsorbent was significantly enhanced after the first regeneration with ozone (TCP removal increased from 52% up to 87%). Elevated adsorption capacity was remained until 8th adsorption cycle, afterwards a dramatic dropdown of TCP removal was observed, possibly because of matrix carbon and pH decrease.</p>
130	4-CP (250 mg/L)	<p><u>Catalytic ozonation</u></p> <ul style="list-style-type: none"> - Catalyst: β-FeOOH nanorods (0.01 g) - O₂/O₃ gas flow rate = 10 mL/min - Ozone concentration in gas phase = 28.24 mg/L - pH = 3.5-10 - Volume = 100 mL 	<p>In the presence of the catalyst, a degradation efficiency of 99% was determined after 40 min ozonation (only 67% when only ozone was used).</p> <p>A maximum of 97% COD was removed using 0.1 g/100 mL catalyst load.</p>
131	PHENOL (100 mg/L)	<p><u>Catalytic and photocatalytic ozonation</u></p> <ul style="list-style-type: none"> - Catalyst: Mn (10%)/TiO₂ (1.5g) - UV light: UV mercury lamp (General Electric, 220-380 nm) - O₂/O₃ gas flow rate = 50 mL/min 	<p>Primary oxidation products: resorcinol, catechol and hydroquinone</p> <p>The product distribution was oriented to more oxidized products (pyruvic, maleic acids and CO₂) when the catalyst was irradiated with UV light.</p>

		<ul style="list-style-type: none"> - Ozone concentration in gas phase = 2.15 mg/L - pH = 3 - Reaction time = 3 h - Temperature = 25 °C - Volume = 100 mL 	The phenol reacted only with ozone and not with hydroxyl radicals. The intermediate ozonation products reacted with both oxidants.
132	2,4-D (10 mg/L)	<u>Ozonation, catalytic ozonation and Photocatalytic ozonation</u> <ul style="list-style-type: none"> - Catalyst: TiO₂ fiber - UV light: UV tube lamp (10 W, 254 nm) - Air/O₃ gas flow rate = 1.0 L/min - Ozone concentration in gas phase = 2 mg/L - pH = not controlled (initial pH = 4.32-4.36) - Reaction time = 2 h - Volume = 7.5 L 	<p>Photocatalytic ozonation with the fiber catalyst was efficient in mineralizing low concentrations of 2,4-D in water (pseudo-first order). The photocatalytic ozonation process was characterized by few aromatic intermediates with low abundance, fast degradations of the aliphatic intermediates despite their relatively large number and dechlorination as a major step.</p> <p>The enhanced mineralization was because of increased ozone dissolution and decomposition, and to reduced electron-hole recombination in the presence of TiO₂ resulting in large amounts of hydroxyl radical formation.</p>
133	2,4,6-TCP (0.5 mg/L)	<u>Catalytic ozonation</u> <ul style="list-style-type: none"> - Catalyst: ZnO (2-10 mg/L; nanometer, submicrometer, micrometer) - Ozone concentration in liquid phase = 2.5 mg/L - pH = 7.5 - Reaction time = 30 min - Temperature = 25 °C - Volume = 2.5 L 	<p>ZnO promoted the formation of hydroxyl radicals and increased the rate of ozone consumption.</p> <p>Under the same conditions, the presence of the catalyst resulted in an increase of the 2,4,6-TCP removal rate.</p> <p>The loss of bound chlorine was observed as one of the first steps of 2,4,6-TCP degradation.</p> <p>The catalytic ozonation by ZnO followed a radical-type mechanism, by which the catalyst was able to initiate or promote hydroxyl radical production.</p> <p>The nano-ZnO particles displayed unique catalytic activity, different from micro-ZnO and submicro-ZnO particles.</p>
134	4-CP (100 mg/L)	<u>Catalytic ozonation</u> <ul style="list-style-type: none"> - Catalyst: MgO (crystal facet of (1 1 1), (1 1 0) and (2 0 0)) - O₂/O₃ gas flow rate = 0.5 L/min - Ozone concentration in gas phase = 5 mg/L - Temperature = 25 °C - Volume = 1000 mL 	<p>The catalytic ozonation on MgO substantially enhanced the removal of 4-CP and TOC, when compared to ozonation only.</p> <p>The mechanism involved hydroxyl radicals in all processes.</p>
135	2,4,6-TCP (100 mg/L)	<u>Catalytic ozonation</u> <ul style="list-style-type: none"> - Catalyst: Ce-Zr loaded metal oxide - Ozone dosage = 14.4 mg/h 	<p>Increased conversion of 2,4,6-TCP was observed at higher pH-values.</p> <p>All reaction products were dechlorinated.</p> <p>Oxidation products: dihydroxyfumaric acid, oxalic acid.</p>

		<ul style="list-style-type: none"> - Reaction time = 5 h - Temperature = 20 °C - Volume = 50 mL 	At pH 11, metal oxide supported Ce-Zr catalysts are found to have good product selectivity for partial oxidation.
136	PHENOL (100 mg/L) 4-CP (100 mg/L)	<p><u>Coupled electro-oxidation/ozonation</u></p> <ul style="list-style-type: none"> - Reactor: upflow bubble column (gas plate diffusor) adapted with two boron doped diamond electrodes (20 cm x 2.5 cm) separated at a distance of 1 cm - Superficial area of electrodes = 50 cm² - Electrolyte = 0.1 M Na₂SO₄ - Current density = 60 mA/cm² for phenol degradation, 30 mA/cm² for 4-CP degradation - Applied potential = 11.25 V - Air/O₃ gas flow rate = 0.05 L/min - Ozone concentration in gas phase = 5 mg/L - pH = 7 - reaction time = 120 min - Temperature = room temperature - Volume = 1L 	<p>Identified intermediates phenol: hydroquinone, benzoquinone, catechol, oxalic acid, maleic acid, succinic acid, fumaric acid, formic acid</p> <p>Identified intermediates 4-CP: hydroquinone, oxalic acid, malonic acid, maleic acid, succinic acid and fumaric acid</p> <p>Complete phenol and 4-CP degradation is achieved in only 60 min and 120 min, respectively. Complete TOC mineralization is only observed during phenol degradation after 100 min treatment.</p> <p>The energy consumption on the basis of TOC removal was calculated to compare with other oxidation processes and the process offering less energy consumption was the coupled process.</p> <p>The main reason for incomplete mineralization was because of the stability of oxalic acid formed as intermediate product during the oxidation process.</p>
137	4-CP (50 mg/L)	<p><u>Photoelectro-peroxone/ZVI process</u></p> <ul style="list-style-type: none"> - Reactor: quartz cylinder (cathode: graphite felt; anode: Platinum sheet) - O₂/O₃ gas flow rate = 0.25 L/min - DC Power supply: 3.000 A, 30 V - UV light: low pressure UVC lamp (4W, 254 nm, 1.51 mW/cm²) - Temperature = 25-28 °C 	<p>The 4-CP degradation by the photoelectro-peroxone/ZVI process had the lowest rate constant, i.e., 0.1809 min⁻¹, compared to metanil yellow, benzotriazole, tetracycline and carmoisine. However, the rate constants are all of the same order of magnitude.</p> <p>For pollutants with large molecule (higher organic carbon, i.e., tetracycline, carmoisine, metanil yellow), the TOC removals were approximately 20-25% lower than others (i.e., 4-CP and benzotriazole).</p>
138	2,4-DCP (100 mg/L) 2,4-D (100 mg/L)	<p><u>Ozonation, Photocatalytic ozonation</u></p> <ul style="list-style-type: none"> - Reactor: planar falling film - UV light: UVA lamps (15 W, 360 nm) - O₂ gas flow rate = 10 L/h - Ozone concentration in gas phase = 130 mg/L - pH = 3.35 and 6.85 - Volume = 0.5 L 	Despite the low mineralization, direct ozonation was an effective process for the decomposition of 2,4-DCP and 2,4-D. The mineralization was enhanced by combining ozonation with photocatalysis, resulting in a nearly complete TOC removal. A similar significant improvement in degradation and mineralization was observed when the plasma under argon atmosphere was combined with Fenton oxidation.
<u>Non-thermal plasma</u>			

		<ul style="list-style-type: none"> - Reactor: dielectric barrier discharge plasma reactor - Ceramic plates (3 mm thickness) - High voltage electrode: self-adhesive aluminum foil - Ground electrode: self-adhesive copper strips - Pulse generator: 21 KV (RMS) at 5 and 20 KHz 	
139	4-chloro-3-methylphenol (50 mg/L)	<u>Ozonation, Catalytic ozonation</u> <ul style="list-style-type: none"> - Reactor: 1.2 L cylindrical glass reactor - Catalyst: Zinc-carbon nanotubes (ZN-CNTs) composite (1-4 g/L) - O₂/O₃ gas flow rate = 200-600 mL/min - Ozone concentration in gas phase = 34 mg/L - (initial) pH = 3-12 - Temperature = 25 °C - Volume = 0.5 L 	<p>The TOC removal rate constant of Zn-CNTs/O₃ was 4.9 times higher compared to single ozonation.</p> <p>The mineralization efficiency increased by increasing the catalyst dosage and oxygen feed rate.</p> <p>Identified intermediates 4-chloro-3-methylphenol: (2Z)-but-2-enedioic acid, malonic acid, formic acid, acetic acid and oxalic acid</p> <p>In the Zn-CNTs/O₃ system, the concentration of small molecular organic acids first increases and afterwards decreased, indicating the oxidation of these intermediates and confirm the enhanced mineralization.</p>

(1) 4-NP: 4-nitrophenol
 (2) 2,4-D: 2,4-dichlorophenoxyacetic acid
 (3) BDDAB: Benzylodexyldimethylammonium bromide
 (4) BNS: Sodium butylnaphthalenesulfonate
 (5) BPA: Bisphenol-A
 (6) DBS: Dodecylbenzenesulfonate
 (7) FAU: Faujasite

Table 7: Overview of recent studies focused on the toxicity and/or biodegradability improvement by ozone-based processes (single substrate solutions)

Reference	Target Pollutant	Conditions of ozone-based oxidation technique	Toxicity/biodegradability assessment	Highlights of the work
<i>Degradation by ozonation</i>				
23	PCP (50 ppm)	<u>Ozonation</u> <ul style="list-style-type: none"> - Reactor: bubble column - Ozone concentration in gas phase = 1% w/w - pH = 5-12 	<u>Biodegradability</u> BOD ₅ and COD measurement <u>Toxicity</u>	The PCP degradation was initiated via direct nucleophilic attack by ozone as predominant pathway, whereas both ozone and secondary hydroxyl radical were likely involved in subsequent degradation of intermediates.

		<ul style="list-style-type: none"> - Reaction time = 45 min - Volume = 4 L 	Colorimetric method based on the reduction of the redox-active dye resazurin by bacterial respiration	<p>The ozonation of PCP resulted in quantitative release of chlorine and multiple intermediates that are increasingly less toxic and biodegradable with time.</p> <p>To detoxify PCP and early toxic intermediates, a reaction time of 10-20 min is needed, afterward intermediates are amenable to biological degradation.</p>
106	4-CP (200 mg/L) 2,4-CP (200 mg/L) PHENOL (200 mg/L)	<u>Ozonation</u> <ul style="list-style-type: none"> - O₂/O₃ gas flow rate = 0.5 L/min - Ozone concentration in gas phase = 23 mg/L - pH = 2-12 - Temperature = 20 °C - Volume = 0.5 L 	<u>Biodegradability</u> BOD ₅ and COD measurements	<p>In the initial step of ozonation, dechlorination of 4-CP and 2,4-DCP occurred, and then the destruction mechanism of these pollutants was similar to the phenols decomposition (at pH 2 and pH 7, formation of phenol and 4-CP was observed during ozonation of 4-CP and 2,4-DCP, respectively).</p> <p>The presence of unsaturated acids at different pH confirmed the cleavage of aromatic ring, but by different mechanisms.</p> <p>Final products at pH 2 & 7: oxalic and formic acid.</p> <p>Final products at alkaline pH: oxalic acid.</p> <p>The effect of pre-ozonation on the biodegradability of phenol, 4-CP and 2,4-DCP was significant and increased at the alkaline pH.</p>
110	CHLOROPHENE (10 mg/L)	<u>Ozonation</u> <ul style="list-style-type: none"> - Ozone volume flow rate = 1.8 L/h - Temperature = 20 °C 	<u>Toxicity</u> Microtox assay (<i>Vibrio fischeri</i>)	<p>The rate constants between ozone and chlorophene in the pH ranges of 3-11 varied from 1.67×10^3 L/(mol.s) at pH 3 to 1.82×10^5 L/(mol.s) at pH 11.</p> <p>The ozonation process generated less toxic intermediate products than the parent pollutant.</p>
140	2-CP (12-65 mg/L) 4-CP (12-65 mg/L) 2,4-DCP (12-65 mg/L) 6-CCR ⁽¹⁾ (12-65 mg/L) 4-CR ⁽²⁾ (12-65 mg/L)	<u>Ozonation</u> <ul style="list-style-type: none"> - Reactor: bubble column - O₂/O₃ gas flow rate = 1.0 L/min - Ozone concentration in gas phase = 0.5 mg/L - pH = 2.5 and 9.5 - Volume = 100 mL 	<u>Toxicity</u> Acute toxicity to <i>Daphnia magna</i>	<p>Second order reaction rate constants for ozonation in acidic media (pH = 2.5) were determined (0.85-1.9 L/(mol.s)).</p> <p>The effect of the pH was found to be strong at pH-values from 3.5 to 8.5.</p> <p>Chloride ion formation was detected during ozonation, which shows the mineralization of CPs.</p> <p>Quinones and hydrogen peroxide were detected as reaction products.</p>

				Ozonation products were much less toxic or even nontoxic to <i>Daphnia magna</i> when compared to the toxic properties of the initial pollutants.
141	2,4-DCP (100 mg/L) NB ⁽³⁾ (100 mg/L)	<u>Ozonation</u> <ul style="list-style-type: none"> - Reactor: batch pilot-scale plant - Ozone dosage = 7.4 g/h and 5.4 g/h for NB and 2,4-DCP, respectively - pH = not controlled - Temperature = 20-25 °C - Volume = 21 L 	<u>Biodegradability</u> <ul style="list-style-type: none"> - BOD₅ and COD measurements - Bioassays (1.5 L, CSTR, pH 7, 1.5 g/L MLSS) 	<p>Stoichiometric coefficients (mol ozone consumed per mol pollutant removed) of the reaction of ozone with NB and 2,4-DCP were approximately 4 and 2, respectively.</p> <p>Pseudo-first order kinetic constants of single ozonation were 3 times higher for 2,4-DCP than NB (the electron withdrawing character of the nitro group depletes the aromatic ring from electron density).</p> <p>Intermediates 2,4-DCP: chlorobenzoquinone and resorcinol</p> <p>For combined ozonation pre-treatment and biological oxidation process, optimum ozone doses were 0.25 g/L and 0.12 g/L for NB and 2,4-DCP, respectively.</p> <p>Highest BOD values were obtained when the pollutants were nearly removed from solution.</p> <p>The coupled system (pre-ozonation and aerobic bioreactor) achieved up to 80% of TOC removal.</p>
142	2-CP (100-200 mg/L) 3-CP (100-200 mg/L) 4-CP (100-200 mg/L)	<u>Ozonation</u> <ul style="list-style-type: none"> - Ozone dosage = 75.2 mg/min - pH = 7 - Volume = 1000 mL 	<u>Toxicity</u> <ul style="list-style-type: none"> - Microtox assay (<i>Photobacterium phosphoreum</i>) - OUR inhibition test (control: glucose) 	<p>The decomposition of 2-CP, 3-CP and 4-CP and the mineralization rates of TOC followed pseudo-first order kinetics during ozonation.</p> <p>Main intermediates 2-CP: 3-chlorocatechol and 2-chloro-2,4-hexadienedioic acid.</p> <p>Main intermediates 3- & 4-CP: 4-chlorocatechol and 3-chloro-2,4-hexadienedioic acid.</p> <p>Ozonation of 2-CP, 3-CP and 4-CP significantly induced new aqueous toxicity if these toxic intermediates were not further ozonated.</p>
143	2,4-DCP (100 mg/L)	<u>Ozonation</u> <ul style="list-style-type: none"> - Reactor: storage tank from which liquid recirculated over venturi-type ozone injector - O₂ gas flow rate = 400 L/h 	<u>Biodegradability</u> <ul style="list-style-type: none"> - BOD (5, 10 and 21 days), COD and TOC measurements - Aerobic bioassays (CSTR; HRT = 12-24 h; non-acclimated and 	<p>After an absorbed ozone dose of 0.12 g/L, the biodegradability (BOD₅/COD) of a 100 mg/L 2,4-DCP solution increased from 0 to 0.25 (average oxidation state: 1.5; no complete dechlorination, indicating the presence of chlorinated intermediates).</p> <p>After the pre-ozonation step, 70% of TOC was removed by an aerobic biological treatment.</p>

			<ul style="list-style-type: none"> - Ozone dosage = 5.4 g/h - pH = not controlled - Reaction time = 30 min - Temperature = 21-25 °C - Volume = 21 L 	acclimated-to-phenol sludge)	
144	2-CP (100-200 mg/L) 3-CP (100-200 mg/L) 4-CP (100-200 mg/L)	<u>Ozonation</u> <ul style="list-style-type: none"> - Reactor: bubble column - O₂/O₃ gas flow rate = 976 mL/min - Ozone concentration in gas phase = 77 mg/L - pH = 7 - Reaction time = 10 min - Volume = 1000 mL 	<u>Toxicity</u> Microtox assay (<i>Photobacterium phosphoreum</i>)	<p>In general, the ozonation of CPs induced new aqueous toxicity.</p> <p>Ozonation of 2-CP revealed higher level of toxicity increase than 3-CP and 4-CP.</p> <p>The increasing toxicity was mainly caused by ozonated intermediates (chlorocatechols, chloromuconic acids and formation of hydroxylated/chlorinated dimeric pollutants).</p> <p>Ozone requirement of detoxifying into a non-toxic solution as following the order 4-CP > 3-CP > 2-CP.</p>	
145	TRICLOSAN (10 mg/L)	<u>Ozonation</u> <ul style="list-style-type: none"> - Ozone concentration in liquid phase = 2 mg/L - pH = 7 - Room temperature 	<u>Toxicity</u> <ul style="list-style-type: none"> - Cytotoxicity: MTT⁽⁴⁾ (tetrazolium dye) test (Chinese hamster ovary cells) - Genotoxicity: alkaline comet assay (Chinese hamster ovary cells) 	<p>Complete triclosan removal was achieved by ozonation.</p> <p>Intermediate products: 2,4-DCP, chlorocatechol, mono-hydroxy-triclosan, di-hydroxy-triclosan</p> <p>2,4-DCP showed lower genotoxic effects than triclosan.</p>	
146	4-CP (465 mg/L) 2,4-DCP (637.5 mg/L) 4-NP (465 mg/L) 2,4-DNP ⁽⁵⁾ (615 mg/L) 4-AP ⁽⁶⁾ (352.5 mg/L) 2,4-DAP ⁽⁷⁾ (292.5 mg/L) ACP ⁽⁸⁾ (420 mg/L) ANP ⁽⁹⁾ (427.5 mg/L)	<u>Ozonation</u> <ul style="list-style-type: none"> - pH = 7.02 - Volume = 1 L 	<u>Biodegradability</u> Aerobic bioassays with non-acclimated municipal activated sludge (based on COD measurements) <u>Toxicity</u> Microtox assay	<p>The oxidant dosage applied to each synthetic solution was equal to the dosage required to achieve a 99% removal of the parent pollutant.</p> <p>Ozonation of purely chloro- and nitrophenols caused significant enhancement of their biodegradability whereas the opposite was observed for aminophenols.</p> <p>Ozonation was found to significantly decrease toxicity for all of the phenolic pollutants.</p>	

147	2-CP (385 mg/L) 4-NP (417 mg/L) 4-CRESOL (325 mg/L)	<u>Ozonation</u> - O ₂ /O ₃ gas flow rate = 0.21 m ³ /h - Ozone concentration in gas phase = 20 g/m ³ - Ozone dosage = 9 g/h - pH = 3-10 - Temperature = 25 °C - Volume = 7 L	<u>Biodegradability</u> BOD ₅ and COD measurements	The removal of COD to effluent standard required long ozonation times and the ozone utilization rate decreased, indicating a costly and unfeasible application. The biodegradability of pre-ozonated phenolic solutions was strongly dependent on the decomposition of phenolic pollutants and accumulation of intermediate products. Using non-acclimated microorganisms, biodegradability (BOD ₅ /COD) of pretreated 4-cresol, 2-CP and 4-NP solutions increased to 0.18, 0.26 and 0.33, respectively (higher ratios were expected if acclimated biomass was used).
Degradation by ozone-based processes				
116	4-CP (130 mg/L)	<u>O₃/UV and O₃/H₂O₂/UV</u> - UV light: low-pressure mercury UV-C lamp (254 nm) - Ozone dosage = 591 mg/h - pH = 3-11 (pH was not controlled during experiments) - Reaction time = 60 min - Temperature = 25 °C - Volume = 0.5 L	<u>Toxicity</u> Microbiotest assay (<i>Daphnia magna</i>)	Complete 4-CP degradation by O ₃ /UV was achieved throughout the pH ranges (increasing the initial pH value up to 9 enhanced the mineralization). The addition of H ₂ O ₂ to the O ₃ /UV process accelerated the decomposition of ozone owing to an increased rate of hydroxyl radical generation. Maximum TOC removal, i.e., 50.1% by O ₃ /H ₂ O ₂ /UV process, was obtained at H ₂ O ₂ concentration of 15 mM (further increase resulted in a decrease of the removal efficiency). The O ₃ /H ₂ O ₂ /UV process achieved an AOX removal efficiency of 96.1% and a toxicity decay of 41.0%. Primary oxidation intermediate products: hydroquinone, benzoquinone, chlorocatechol and phenol
148	PCP (50 mg/L) ALACHLOR (50 mg/L) ATRAZINE (saturated solution) CHLORFENVINFOS (50 mg/L) DIURON (saturated solution)	<u>Photocatalytic ozonation</u> - Catalyst: Fe (2 mg/L FeSO ₄ ·7H ₂ O) for photo-Fenton/ozone; TiO ₂ (250 mg/L) for photocatalytic ozonation - Extra oxidant: H ₂ O ₂ for Photo-Fenton/ozone - UV light: black light (6 W, Philips)	<u>Toxicity</u> BioTox (<i>Vibrio fischeri</i> luminescence inhibition)	The degradation processes followed first and zero order kinetics, when photo-Fenton/ozone and photocatalytic ozonation were applied, respectively. The best results of pesticide degradation were obtained when the photo-Fenton/ozone combination was applied. The pesticide solutions became detoxified after photo-Fenton/ozone treatment, except for atrazine and alachlor for which no detoxification was achieved (in the case of alachlor, the toxicity increased with increasing irradiation time).

	ISOPROTURON (50 mg/L)	- Ozone dosage = 1.6 g/h - pH = 3 (except PCP: pH 7) - Temperature = 25 °C - Volume = 200 mL		
149	<i>Phenolic acids</i> SYRINGIC ACID ⁽¹⁰⁾ (100 mg/L) VANILLIC ACID ⁽¹¹⁾ (100 mg/L) 3,4,5-TMBA ⁽¹²⁾ (100 mg/L) 3,4-DMBA ⁽¹³⁾ (100 mg/L) 3,4-DHBA ⁽¹⁴⁾ (100 mg/L) 4-HBA ⁽¹⁵⁾ (100 mg/L)	<i>Ozonation and catalytic ozonation</i> - Catalyst: Mn-Ce-O 70/30 and Fe ₂ O ₃ -MnO _x N-150 (10 g/L) - O ₂ /O ₃ gas flow rate = 500 mL/min - Ozone concentration in gas phase = 40 mg/L - pH = 3.4 - atmospheric pressure and room temperature - Volume = 500 mL	<i>Biodegradability</i> - BOD ₅ and COD measurements - Respirometry (control: acetic acid; activated sludge from an industrial winery) <i>Toxicity</i> - Respirometry (control: acetic acid; activated sludge from an industrial winery) - Luminescence light inhibition (<i>Vibrio fischeri</i>)	The use of solid catalysts in the ozone-based systems resulted in a high improvement of both organic charge removal and biodegradability level. Based on the COD removal results, the catalytic ozonation over N-150 catalyst achieved the best results, but further treatment was necessary. All considered oxidation techniques were able to completely detoxify the synthetic wastewater. Catalytic ozonation over Mn-Ce-O 70/30 improved the biodegradability level from 0% to 61%.
⁽¹⁾ 6-CCR: 6-chloro- <i>meta</i> -creasol ⁽²⁾ 4-CR: 4-chlororesorcinol ⁽³⁾ NB: Nitrobenzene ⁽⁴⁾ MTT: Methyl tetrazolium ⁽⁵⁾ 2,4-DNP: 2,4-dinitrophenol ⁽⁶⁾ 4-AP: 4-aminophenol ⁽⁷⁾ 2,4-DAP: 2,4-diaminophenol ⁽⁸⁾ ACP: 2-amino-4-chlorophenol		⁽⁹⁾ ANP: 2-amino-4-nitrophenol ⁽¹⁰⁾ Syringic acid: 4-hydroxy-3,5-dimethoxybenzoic acid ⁽¹¹⁾ Vanillic acid: 4-hydroxy-3-methoxybenzoic acid ⁽¹²⁾ 3,4,5-TMBA: 3,4,5-trimethoxybenzoic acid ⁽¹³⁾ 3,4-DMBA: 3,4-dimethoxybenzoic acid (veratric acid) ⁽¹⁴⁾ 3,4-DHBA: 3,4-dihydroxybenzoic acid (photocatechuic acid) ⁽¹⁵⁾ 4-HBA: 4-hydroxybenzoic acid		

Table 8: Overview of recent studies focused on the degradation of chlorophenolic pollutants present in multiple substrate solutions

Reference	Target Pollutant	Conditions of ozone-based oxidation technique	Highlights of the work
<i>Degradation by ozonation</i>			
112	4-NP (139-195 mg/L)	<i>Ozonation</i> - Reactor: bubble column - Matrix pollutant: <i>t</i> -butanol (0.017-1.667 g/L) - Superficial gas velocity = 0.0164 m/s	pH determined the relative production rates of hydroquinone and 4-nitrocatechol along the reaction path. An optimal concentration of <i>t</i> -butanol was observed at which production of the analyzed intermediates was slowest, indicating that radicals played an important role in 4-NP ozonation at low pH.

		<ul style="list-style-type: none"> - Ozone concentration in gas phase = 2.5-7.0 mg/L - Ozone concentration in liquid phase = 0-0.5 mg/L - pH = 2-10 - Temperature = 20 °C 	
113	4-CP (25.6 mg/L) 2,4-DCP (32.6 mg/L) 2,4,6-TCP (39.4 mg/L) 2,3,4,6-TeCP (46.2 mg/L) TETRACHLOROCATECHOL (49.6 mg/L) 4-CHLOROGUAIACOL (31.2 mg/L)	<u>Ozonation</u> <ul style="list-style-type: none"> - Matrix component: <i>t</i>-butanol (740 mg/L) - pH = 2-9 - Temperature = 20 °C - Volume = 500 mL 	<p>The reaction between the oxidant and the organic followed overall second order kinetics, and more specifically, of first order with respect to both reactants.</p> <p>The second-order rate constants increased with the pH as a consequence of the increase in the degree of deprotonation of the dissolved CPs (i.e., from 10³ L/(mol.s) to 10⁹ L/(mol.s) for different CPs).</p> <p>The specific rate constants obtained are significantly higher for the ozonation of the dissociated species.</p>
150	PHENOL (0.25-1 mg/L) 4-BROMOPHENOL 2,4,6-TCP 2,4-DIBROMOPHENOL 2,4,6-TRIBROMOPHENOL	<u>Ozonation</u> <ul style="list-style-type: none"> - Matrix pollutant: salt (as seawater) - Chlorination of phenol in seawater - Ozone stock solution: 30-50 mgO₃/L - Ozone dosage = 5-10 mg/L - pH = 7.0 - Reaction time = 30 min - Temperature = 22 °C - Volume = 500 mL 	<p>Ozone was powerful on degrading DOM with high MW (200-1000 Da) than that with lower MW (20-100 Da).</p> <p>Pre-ozonation with an initial dose of 5 mgO₃/L significantly minimized halophenols formation in seawater samples during chlorination.</p>
Degradation by ozone-based processes			
151	2-CP (40 mg/L)	<u>Ozonation and O₃/UV</u> <ul style="list-style-type: none"> - Matrix pollutant: <i>t</i>-butanol (37-740 mg/L) - UV light: low-pressure mercury-vapor UV lamp (254 nm) - O₂/O₃ gas flow rate = 34 L/h - Ozone concentration in gas phase = 21 mg/L - pH = 3-11 - Reaction time = 2 h - Temperature = 25 °C - Volume = 2 L 	<p>Almost complete decomposition of 2-CP was accomplished in 20 min by both oxidation techniques.</p> <p>The release of chloride ions was observed early during the decomposition reaction, followed by the mineralization of organic intermediates to form carbonate ions.</p> <p>The presence of <i>t</i>-butanol promoted the decomposition of 2-CP in both processes. In alkaline solutions excessive dosages decreased the reaction rates.</p> <p>The behavior of 2-CP in the presence of <i>t</i>-butanol during both ozonation and O₃/UV was described by pseudo-first order kinetics.</p>

152	2,4,6-TCP (39.5-73.0 mg/L) ATRAZINE (30 mg/L)	<u>Ozonation and O₃/H₂O₂</u> <ul style="list-style-type: none"> - Matrix pollutant: humic acid (2.4-56.1 mg/L) - O₂/O₃ gas flow rate = 1 L/min - H₂O₂ concentration = 516 mg/L - pH = 2-8.2 - Reaction time = 10 min - Temperature = 23 °C - Volume = 500 mL 	<p>The rate constant of 2,4,6-TCP degradation by ozone increased one order of magnitude by increasing the pH from 2 to 7.5, owing to the higher reactivity of the dissociated TCP and the contribution of ozone radical oxidation.</p> <p>At pH 7.5, 10 min of ozonation achieved a 90% TCP degradation, which corresponded to the release of approximately 2 mol Cl⁻ per mol TCP.</p> <p>The presence of H₂O₂ did not significantly increase the TCP degradation but increased the overall dechlorination to 2.7 mol Cl⁻ per mol TCP.</p> <p>The presence of humic acid enhanced the degradation rate of TCP at low relative humic acid concentrations (< 0.6 g humic acid per g TCP), but reduced the rate at higher humic acid concentrations.</p>
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Table 9: The toxicity and/or biodegradability improvement of multiple substrate solutions by ozone-based processes

Reference	Target Pollutant	Conditions of ozone-based oxidation technique	Toxicity/biodegradability assessment	Highlights of the work
<i>Degradation by ozonation</i>				
115	2,4-DCP (100 mg/L) 2,4-D (100 mg/L) 2,4,5-T (100 mg/L)	<u>Ozonation</u> <ul style="list-style-type: none"> - Matrix pollutant: salt - O₂/O₃ gas flow rate = 0.4 m³/h - Ozone concentration in gas phase = 15 g/m³ - pH = 8 - Reaction time = 30 min - Temperature = 30 °C - Volume = 2 L 	<u>Biodegradability</u> Bioreactor, with activated sludge (pH 7, 30 °C)	<p>All phenolic pollutants disappeared with ozone oxidation, and consequently transformed into various organic acids.</p> <p>The TOC value decreased by increasing the ozonation time up to half of the initial value.</p> <p>The activated sludge treatment after ozonation was effective to degrade the intermediate products (based on TOC removal).</p>
153	2,4-DCP (100 mg/L)	<u>Ozonation</u> <ul style="list-style-type: none"> - Matrix pollutant: sodium acetate (320 mg/L) + glucose (235 mg/L) & sodium acetate (320 mg/L) + Baby milk powder (159 mg/L) 	<u>Biodegradability/Toxicity</u> Respirometry, using non-acclimated and pre-exposed (not fully acclimated) activated sludge	<p>The inversely proportional relationship between the 2,4-DCP degradation and the chlorine release was clearly observed.</p> <p>Complete mineralization was not achieved, implying the production of dehalogenated intermediates.</p> <p>The presence of biodegradable organic matter results in a decreased pseudo-first order degradation rate. The extent</p>

		<ul style="list-style-type: none"> - O₂/O₃ gas flow rate = 400 L/h - Ozone dosage = 6 g/h - pH = 9 - Reaction time = 2, 5 and 10 min - Volume = 2 L 		<p>of the decrease depends on the nature of the biodegradable organic matter.</p> <p>A long lag phase and low maximal Specific Oxygen Uptake Rate (SOUR) value were observed as most important toxicity effects.</p> <p>Because of ozonation, the lag phase was significantly reduced and the maximal SOUR value increased after elongating the ozone reaction time. Also, the biodegradable COD fraction increased.</p>
154	2,4-DCP (100 mg/L)	<p><u>Ozonation</u></p> <ul style="list-style-type: none"> - Matrix pollutant: artificial wastewater containing glucose (C/N/P = 100/14.4/1.9; monovalent over polyvalent cation ratio = 2.02) - O₂/O₃ gas flow rate = 200 L/h - Ozone dosage = 8 g/h - pH = 9 - Reaction time = 30 min - Volume = 40 L 	<p><u>Biodegradability</u></p> <p>Bioreactor, with activated sludge (pH 7.8, 20 °C)</p>	<p>An increase of the effluent COD concentration was observed when pre-ozonated wastewater was used as influent of the bioreactor. The intermediate ozonation products of 2,4-DCP were not completely biodegradable. Furthermore, a higher biomass concentration and enhanced sludge settleability were observed.</p> <p>Without ozone pretreatment, the 2,4-DCP was completely biodegraded. However, the effluent was not clear because of small particles and a hampered settling of the sludge was observed.</p>
Degradation by ozone-based processes				
155	2,5-DCP (500 mg/L)	<p><u>Ozonation, O₃/UV</u></p> <ul style="list-style-type: none"> - Reactor: bubble column glass reactor (height = 58 cm; inner diameter = 8.5 cm; fine pore diffuser of 100-µm diameter) - Matrix pollutant: municipal wastewater (TOC = 115 mg/L; COD = 250 mg/L; BOD₅ = 198 mg/L; TSS = 130 mg/L; TDS = 548 mg/L; NO₃⁻-N 	<p><u>Biodegradability</u></p> <ul style="list-style-type: none"> - BOD₅ and COD measurements <p><u>Toxicity</u></p> <ul style="list-style-type: none"> - Respirometry (ISO 8192:2007; control: synthetic sewage solution) 	<p>Main intermediates: 2,5-dichloro-1,4-benzenediol, 3,6-dichloro-benzene-1,2-diol, catechol, 4-chloro-1,3-benzenediol, phenol, 4-chloro-1,2-benzenediol, 3-CP, pentanoic acid</p> <p>Complete degradation of 2,5-DCP by ozone and O₃/UV occurred after 60 min and 40 min, respectively.</p> <p>The 2,5-DCP degradation and dechlorination proceeded simultaneously.</p> <p>The pseudo-first order rate constants obtained for O₃/UV were more than 1.5 times faster than the ones for ozonation.</p>

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- = 10 mg/L; PO_4^{3-} = 6 mg/L; Cl^- = 30 mg/L)
- UV-light: Pen-Ray Uv lamp (4 nW/cm² at a distance of 2.54 cm)
- O_3/O_2 gas flow rate = 36 L/h
- Ozone concentration in gas phase = 37 mg/L
- Ozone dosage = 22 mg/min
- pH = 7-8 (neutral)

The BOD₅/COD ratio increased from 0.19 to 0.46 (after 40 min) and 0.5 (after 30 min) by ozonation and O_3/UV , respectively.

Both treatments of 40 min and 60 min created intermediate products which were not sufficiently toxic to calculate the EC₅₀-value (initial EC₅₀ = 57 mg/L). Therefore, these products were less toxic than the parent compound.

The presence of other organic and inorganic matter had a positive effect on the oxidation of 2,5-DCP.

alkaline pH.²² Moreover, ozonation was more reactive with higher substituted chlorophenols whereas hydroxyl radicals reacted faster with chlorophenols having lower number of chlorine atoms. The reaction between ozone and chlorophenolic substances follows overall second order kinetics and is more specifically of first order with respect to both reactants.^{113,117,140} Benítez *et al.* reported the second order rate constants of PCP ozonation at pH 2.5 and pH 9 to be 0.67×10^3 L/(mol.s) and 3.15×10^7 L/(mol.s), respectively.¹¹⁸ In an earlier study investigating the ozone degradation of lower substituted chlorophenols (4-CP, 2,4-DCP, 2,4,6-TCP and 2,3,4,6-Tetrachlorophenol), Benítez *et al.* reported a larger increase in the second order rate constants (from 10^3 L/(mol.s) to 10^9 L/(mol.s)) by increasing the pH (from pH 2 to pH 9, respectively).¹¹³ During the ozonation degradation of chlorophenols, the rate constants varied from 1.72×10^3 L/(mol.s) at pH 3 to 1.82×10^5 L/(mol.s) at pH 11.¹¹⁰ Again, these results confirmed the faster reaction of chlorophenols with ozone in an alkaline environment because of the deprotonation of the dissolved chlorophenols. Despite the overall second order kinetics, many studies describe the ozone-based degradation of chlorophenols, COD and/or TOC by pseudo-first order kinetics with respect to the chlorophenol concentration.^{79, 22,119,122-125,141-142,151} In the aforementioned studies, the oxidizing agent was present in excess, hence the concentration could be assumed constant. Moreover, Benítez *et al.* reported the stoichiometric ratio for the oxidation of PCP by ozone to be 1.83 mol ozone consumed per mol PCP reacted.¹¹⁸ For the ozone degradation of 2,4-DCP, the stoichiometric coefficient was approximately 2 mol ozone per mol 2,4-DCP removed as determined by Contreras *et al.*¹⁴¹ However, ozone dosages based on mass are also reported in the literature. For example, Manojlovic *et al.* applied different ozone dosages for different chlorophenols, i.e., 2.6 mgO₃/mgC, 2.0 mgO₃/mgC and 2.0 mgO₃/mgC for phenol, 4-CP and 2,4-DCP, respectively.¹²⁰ During the decomposition reaction, the early release of chlorophenol ions was observed by Hong and Zeng, Poznyak and Vivero, and Wang, Peira *et al.* and Graham *et al.*, indicating that dechlorination is one of the first steps in the degradation pathways.^{23,106,126,151-152} Graham *et al.* determined a chlorophenol release of approximately 2 mol Cl⁻ per mol 2,4,6-TPC.⁹⁷ According to Benítez *et al.*, the dechlorination is enhanced by the presence of more chlorine atoms substituted in the aromatic ring.²² Poznyak and Vivero observed the dechlorination of 4-CP and 2,4-DCP, resulting in the formation of phenol and 4-chlorophenol, respectively.¹⁰⁶ However, complete dechlorination is not always achieved, indicating the presence of chlorinated intermediates such as chlorinated aliphatic structures.^{114,143} Shang *et al.* identified chlorocatechols, chloromuconic acids and chlorinated dimeric pollutants as intermediate products formed during the ozonation of monochlorophenols.¹⁴⁴ Also, the ozonation of PCP resulted in the formation of tetrachlorocatechol, tetrachlorohydroquinone and tetrachloro-p-benzoquinone.¹¹⁸ Furthermore, Shang *et al.* reported 3-chlorocatechol and 3-chloro-2,4-hexadienedioic acid as main intermediates in the ozonation of 2 CP, whereas the ozonation of 3-CP and 4-CP resulted in the formation of 4-chlorocatechol and 3-chloro-2,4-hexadienedioic acid.¹⁴² 2,4-DCP and chlorocatechol were

identified as intermediate species during the ozonation of triclosan.¹²⁷ Finally, hydroquinone, benzoquinone, chlorocatechol and phenol were identified as intermediate products of primary oxidation from the treatment of 4-CP by O₃/UV and O₃/H₂O₂/UV processes.¹¹⁶

Further treatment of the chlorophenols and intermediate products was accompanied by a decrease in pH resulting in acidic oxidation products.¹¹⁴ Also, the colour of the reaction mixture changed, indicating the formation of new chromophores, most likely quinones and their derivatives. Towards the end of the overall treatment, the colour of the mixture again disappeared. As oxidation end-products, oxalic acid, maleic acid, fumaric acid, glyoxylic acid and formic acid were observed by Beltrán *et al.*¹²³ Poznyak and Vivero observed the production of oxalic and formic acid during the ozonation of phenol, 4-CP or 2,4-DCP at pH 2 and pH 7, whereas only oxalic acid was identified after ozonation at alkaline pH.¹⁰⁶ The presence of unsaturated acids at different pH confirmed the cleavage of the aromatic ring, but by different mechanisms.

Only few studies focused on the effect of other substances on the ozone degradation of chlorophenolic pollutants. The influence of the presence of *t*-butanol in chlorophenol-containing solutions was investigated by Kuosa *et al.*, Benítez *et al.* and Ku and Wang.^{112-113,151} Benítez *et al.* used *t*-butanol as a hydroxyl radical scavenger to determine the rate constants for the direct ozonation of chlorophenols.¹¹³ Ku and Wang observed an enhancement of the decomposition of 2-CP by both ozonation and O₃/UV processes because of the presence of *t*-butanol.¹⁵¹ However, in an alkaline environment, excessive dosages of *t*-butanol decreased the reaction rates. Furthermore, during the ozonation of 4-NP at low pH, the effect of *t*-butanol on the production of intermediates was investigated by Kuosa *et al.*¹¹² Also the presence of humic acid influenced the degradation rate of 2,4,6-TCP.¹⁵² At low relative humic acid concentrations (< 0.6 g humic acid per g TCP) the reaction rates were enhanced. However, the opposite was observed at higher humic acid concentrations. The combined ozone oxidation and activated sludge treatment of 2,4-DCP, 2,4-D and 2,4,5-T with or without artificial seawater containing NaCl, MgSO₄, MgCl₂, CaCl₂ and KCl was examined by Nakamura *et al.*¹¹⁵ Ding *et al.* used pre-ozonation treatment to control halophenol formation in seawater during chlorination.¹⁵⁰ These few publications indicated the importance of degradation studies of pollutants present in a mixture of different components. However, the focus of most of the studies on the ozonation of chlorophenolic pollutants is on the evaluation of degradation levels or the identification of reaction intermediate products. In this context, an opinion paper by Oller *et al.* reports the need for more studies on individual and global degradation efficiencies by AOPs in multiple substrate solutions.³

4.2. Toxicity and biodegradability assessment of intermediate products

The complete mineralization of recalcitrant organic matter by AOPs into CO₂ and inorganic end-products has been reported to be only obtained after long reaction times, even when the half-

life time of the original contaminant was very short.¹⁵⁶⁻¹⁵⁸ Therefore, the determination of the original pollutant concentrations is only a partial measure of the efficiency of treatment.¹¹⁴ Moreover, the formation of both early toxic intermediates (such as chlorocatechols) and low molecular weight (organic acids) or hydroxylated pollutants was reported.^{23,144} The latter intermediate products were considered to be less toxic and more biodegradable than the parent pollutants.^{115,140,143} In the context of coupling pre-ozonation with subsequent biological treatment, an appropriate toxicity and biodegradability assessment of the intermediate products and additional wastewater characteristic is necessary. Unfortunately, an evaluation of the toxicity and/or biodegradability of the intermediates is not always discussed. In this literature overview, only 37% of the studies included toxicity and/or biodegradability measurements. However, the importance of toxicity and biodegradability tests is crucial, as was pointed out by Oller *et al.*³ Currently no single standard parameter or method is suitable to assess the toxicity of pretreated phenolic solutions. The genotoxicity and cytotoxicity of triclosan and intermediate ozonation products were determined by Chen *et al.* using an alkaline comet assay and MTT (3-(4,5-dimethylthiazol-2-yl)-5-*tert*-butyl-4-methylthiazolium bromide) test on Chinese hamster ovary cells, respectively.¹⁴⁵ In many cases, (acute) microtox assays were applied using specific bacteria. Adams *et al.* used microtox assays to conclude that ozonation significantly decreases the toxicity of solutions containing chloro-, nitro-, and aminophenols.¹⁴⁶ The IC_{50} -value of 2-CP, 4-CP and 2,4-DCP, i.e., 17.95 mg/L, 8.07 mg/L and 2.68 mg/L, respectively, was determined using *Daphnia magna*.¹⁴⁰ The same organisms were used by Peternel *et al.* to determine a toxicity decay of approximately 41% after $O_3/H_2O_2/UV$ oxidation of 130 mg/L 4-CP.¹¹⁶ An increased toxicity based on the growth of *Photobacterium phosphoreum* was observed after ozonation of monochlorophenols.¹⁴⁴ With the exception of atrazine and alachlor, Farré *et al.* observed lower toxicity towards *Vibrio fischeri* after photocatalytic ozonation of pesticide solutions.⁹⁵ Based on microtox assays using the same bacteria, Benítez *et al.* concluded that the generated intermediate products were less toxic than the parent pollutant (chlorophene).¹¹⁰ A colorimetric method based on the reduction of the redox-active dye resazurin by bacterial respiration was used to evaluate the detoxification of PCP and early toxic intermediates by ozonation.²³ Shang *et al.* used both Microtox assays on *Photobacterium phosphoreum* and Oxygen Uptake Rate (OUR) inhibition tests on activated sludge to evaluate the acute toxicity of pure CPs and ozonated solutions.¹⁴² The OUR inhibition test, based on respirometry, was also applied by Martins and Quinta-Ferreira and Alexander and Ramirez-Cortina, and used a bacterial consortium representative for the aerobic biological treatment.^{149,155} Therefore, this method is particularly suitable for evaluating the detoxification caused by pre-ozonation. Several authors assessed the enhancement of biodegradability of phenolic solutions based on the evolution of the BOD_5/COD ratio during ozonation.^{23,106,141,143,147,149,155} This ratio provides an approximate index of the proportion of organic substances that

are biodegradable under aerobic conditions for a predetermined period of time.³ Whenever the BOD_5/COD ratio exceeded a value of 0.3-0.4, the pre-treated wastewater is considered to be degradable in an aerobic biological treatment.¹⁵⁸⁻¹⁶¹ Hsu *et al.* reported that pre-ozonation of chlorophenolic pollutants was found to enhance the effectiveness of a subsequent biological treatment.¹⁴⁷ Poznyak and Vivero stated that the effect of the preliminary ozonation to the biodegradability of phenol, 4-CP and 2,4-DCP is significant and increases at alkaline pH.¹⁰⁶ Otherwise, respirometric measurements (short analysis) and long-term biodegradability assays were used to evaluate the behaviour of activated sludge when pre-ozonated wastewater was added.^{115,141,143,146,149} Martins and Quinta-Ferreira determined the oxygen consumption rate during biodegradation of a pre-treated synthetic phenolic mixture and compared this value with the OUR-value obtained during degradation of acetic acid, which is considered as readily biodegradable.¹⁴⁹ Based on respirometry, the ozonation and catalytic ozonation realized an increased biodegradability from 0% to 18% and to 61%, respectively. A significant enhancement of biodegradability was not observed by evaluating BOD_5/COD ratios. Based on SOUR-profiles, Van Aken *et al.* identified a long lag phase and low maximal SOUR value as most important toxicity effects of 2,4-DCP present in a multiple substrate solution.¹⁵³ The lag phase was significantly reduced and the maximal SOUR value was increased by increasing the ozone reaction time. Furthermore, by pre-treating the wastewater containing 2,4-DCP, sodium acetate and glucose for 10 min of ozone reaction time, the ratio of the biodegradable COD fraction increases from 78% to 90% for non-acclimated biomass and from 77% to 85% for pre-exposed biomass. Indeed, the respirometric measurements and the derived OUR-values provide useful information to evaluate a process efficiency. Eslami *et al.* even calculated a biodegradability and toxicity value by comparing OUR values of a wastewater sample with a high biodegradable compound, such as acetic acid.¹⁶² To respond to acclimation effects of biomass in the long term, Adams *et al.* investigated the pre-ozonation of 4-CP and 2,4-DCP and observed an increase in COD removal by 50% during biodegradation of the pre-ozonated solutions.¹⁴⁶ The change of TOC of ozonated endocrine-disrupting chemicals (2,4-DCP, 2,4-D and 2,4,5-T) in water after activated sludge treatment was observed by Nakamura *et al.*¹¹⁵ They observed no TOC reduction during biotreatment of 2,4-DCP without pre-ozonation, whereas, after 30 min ozonation, the TOC reduction caused by the activated sludge treatment increased up to 67%. The effectiveness of ozonation as pre-treatment of DCP solutions was shown by feeding the pre-ozonated effluent to aerobic reactors, achieving up to 80% of TOC removal. Also other ozone-based systems (O_3/H_2O_2 , O_3/UV , $O_3/UV/H_2O_2$ and $O_3/UV/Fe$) were investigated, but, compared to single ozonation, no significant improvement was achieved regarding biodegradability enhancement. Van Aken *et al.* investigated the effect of pre-ozonation prior to a biological treatment experimentally by using a pilot-scale reactor.¹⁵⁴ Firstly, the activated sludge was adapted to a glucose based artificial wastewater. After stabilization, the wastewater was spiked with 2,4-DCP and ozonated before

introduced to the bioreactor. The effluent COD increased, indicating that the ozonation intermediates were not completely biodegradable. However, the biomass concentration was increased and the sludge settleability improved. Recently, a few authors used live/dead staining in long-term biodegradability studies to evaluate the biomass viability.¹⁶³⁻¹⁶⁴ However, advanced oxidation processes applied in these studies were not ozone-based.

To eliminate the toxicity attributed to ozonation byproducts, Bourgin *et al.* suggests biological filtration, i.e., biological activated carbon (BAC), as a sufficient post-treatment ozonation.¹⁶⁵ Indeed, ozonation can cause the increment of some toxicity endpoints. Furthermore, the carbon-bed life of the BAC can be prolonged by converting recalcitrant organics to biodegradable organics by pre-ozonation.¹⁶⁶ In this BAC process, bacteria are trapped within the activated carbon, causing the in situ biological regeneration of sorption sites. When recalcitrant organics are degraded into biodegradable organics, the attached microorganisms then mineralize them before they can occupy adsorption sites on the activated carbon.¹⁶⁷

In general, BAC processes are applied to treat secondary effluent containing organic pollutants in the range of 1 ng/L to 200 µg/L.¹⁶⁸⁻¹⁷⁰ Speitel *et al.* studied the biodegradation of substituted phenols in granular activated carbon columns and concluded that para-nitrophenol and 2,4-DCP are readily biodegraded at influent concentration less than 10 µg/L. PCP was also found to be biodegradable, however, at a lower rate. Moreover, a substantial fraction of 2,4-DCP was irreversibly adsorbed, indicating negligible bioregeneration of the activated carbon. Remarkably, the combination of ozonation and the BAC process is widely used for drinking water production, however, only rarely investigated for the treatment of secondary effluents.¹⁶⁹ Only a few studies are dedicated to demonstrate the feasibility of ozone-based techniques in combination with BAC. The conversion of biorecalcitrant pollutants, such as phenol, benzoic acid and aminobenzoic acid by a laboratory scale pre-ozonation and lifted moving-bed BAC treatment was investigated by Lin *et al.*¹⁷¹ Compared to GAC adsorption, the ozone-BAC system can be used 4-5 times longer. Furthermore, the enhanced COD removal capability of the biofilm is caused by both the increase of the COD removal capability of the acclimated bacteria and the species succession of bacteria in the biofilm. The removal of micro-pollutants, such as 2,4-dichloro-benzaniline, 2,7-bis(1,1-dimethylethyl) phenol and 4-bromo-3-chloroaniline, by ozonation, O₃/UV/TiO₂/UV/O₃ in combination with BAC was demonstrated for secondary effluents of a municipal sewage treatment plant in Beijing city (China).^{169,172} Remarkably, the organic matter with a molecular weight (MW) < 10000 Da were mostly removed by TiO₂/UV/O₃. Also UV/O₃ obtained a high removal rate in the MW range. However, ozonation showed the best decomposition for the organic matter with a MW > 10000 Da. The TiO₂/UV treatment resulted in the disappearance of some aromatic compounds, including 4-bromo-3-chloroaniline, 2,4-dichlorobenzaniline and 3,5-dimethoxy-acetophenone and the formation of small molecules, e.g., 1,3-cyclohexanediamine, octadecanamide, *tert*-butyldimethylsilanol and phenol. The

organics were mostly reduced by the subsequent BAC treatment. Gerrity *et al.* examined an alternative treatment strategy, including ultrafiltration/sand filtration, ozone/H₂O₂ and BAC, for indirect potable reuse of the secondary effluent from the Reno-Stead Water Reclamation Facility.¹⁶⁸ The ozone/H₂O₂ and BAC processes were extremely effective in reducing the concentrations of trace organic contaminants, including bisphenol A, octylphenol, triclosan and ibuprofen. The feasibility of the treatment train was demonstrated in pilot scale installations. Nevertheless, specific studies covering the removal of chlorophenols by ozone (or ozone-based techniques) and BAC as a last finishing step are limited. Additional work on this topic is recommended.

4.3. Novel trends

The efficiency of ozone-related processes is limited by the low dissolved ozone concentration in aqueous solutions.¹⁵¹ An intense contact between gas and liquid phase is necessary to enhance the mass transfer of ozone. Traditionally, the contact between both phases is mostly achieved in a bubble column.^{23,114,127-128,140,144} The properties of the liquid and the type of diffuser at the bottom of the column determine the size of the bubbles, and consequently the contact area between gas and liquid phase. Also, venturi-like injectors are used.¹⁴³ As a first fluid (water) traverses the constricted throat of the venturi, a pressure drop is created, which implicates the draw-up of a second fluid (ozone gas). Chedeville *et al.* compared different gas/liquid contactors and concluded that both volumetric mass transfer coefficients and gas retention rates of the venturi were higher, compared to the bubble column.¹²¹ Recently, the use of a microchannel reactor for the ozonation of PCP in solution was investigated by Ren *et al.*¹¹⁹ Typical Taylor flow patterns were observed under the investigated experimental conditions, resulting in an excellent performance for the ozone mass transfer efficiency. Moreover, apparent *pseudo*-first order reaction rate constants between 2.10 s⁻¹ and 1.13 s⁻¹ were reported.

The application of ozone as chemical oxidant for the degradation of chlorophenolic pollutants has already been discussed. However, this oxidation technique did not achieve a complete mineralization of the water pollutants. To overcome this drawback, different approaches are discussed in literature. Aziz *et al.* investigated the efficiencies of ozonation, photocatalysis and non-thermal plasma and compared these techniques based on energy yield and degree of mineralization.¹³⁸ They concluded that the appropriate method for extensive mineralization should be photocatalytic ozonation or a plasma assisted destruction. Zhang *et al.* investigated the adsorption of 2,4,6-TCP on a high-silica zeolite adsorbent, which was regenerated by ozonation.¹²⁹ After the first regeneration with ozone, the TCP adsorption was significantly enhanced (from 52% up to 87% TCP removal). The improved adsorption capacity remained until the 8th adsorption cycle. Afterwards a dramatic decrease of TCP removal was observed. The authors ascribed this to a decrease in pH and the presence of intermediate products, which interfered with the TCP adsorption. Another approach to enhance the mineralization of chlorophenolic pollutants, is catalytic or photocatalytic

ozonation using metal oxides. These ozone-based oxidation techniques are considered to be environmentally friendly and highly effective.^{125,130} The combination of ozone and UV-light accelerates the decomposition rate of different chlorophenols with an extremely high rate constant for 4-CP.²² Both catalysts TiO_2 and Fe(II) ions improved the reaction efficiency of photocatalytic ozonation of 2,4-D.¹²⁶ Villaseñor *et al.* investigated the manganese-supported TiO_2 catalyst for degradation of phenol.¹³¹ The catalytic behaviour in absence of irradiation did not show significant changes for a manganese-supported catalyst. However, irradiated systems achieved an increased phenol degradation, resulting in CO_2 and small organic acids as final products. Several studies reported the improvement of mineralization using TiO_2 as photocatalyst during ozonation of chlorophenolic pollutants.^{123-124,127,132,148} Other catalysts such as $\beta\text{-FeOOH}$ nanorods, MgO , Ni containing fly ash and sawdust, ZnO , and Zn-C nanotubes were also reported to improve the overall degradation efficiency.^{125,130,133-134,139} Nowadays, bimetallic catalysts are increasing in interest because of their synergistic behaviour which makes catalysts more effective and durable.¹³⁵ Martins and Quinta-Ferreira used the lanthanide cerium (Ce) combined with manganese (Mn) to catalyze the ozonation of different phenolic acids.¹⁴⁹ The use of the catalyst Mn-Ce-O in the ozone-based system resulted in a substantial improvement of both organic removal and biodegradability level. An increased conversion and good product selectivity for partial oxidation was observed during ozonation of 2,4,6-TCP catalysed by a Ce-Zr loaded metal oxide.¹³⁵ The lanthanide metals are characterized by their specific electronic structure (4f^x5d^y), resulting in the formation of labile oxygen vacancies with a relatively high mobility of bulk oxygen species.¹⁷³ Recently, electro-oxidation was coupled with ozonation for the degradation of phenol and 4-CP by Hurtado *et al.* Interestingly, less energy (on the basis of TOC removal) was consumed compared to other oxidation processes. The photoelectron-peroxone/zero valent iron process was studied for several emerging pollutants including 4-CP and showed effective results for the treatment of actual industrial wastewater in terms of COD, TOC and color.¹³⁷

5. Conclusion

Both biological and ozone-based oxidation processes are already well defined for the degradation of chlorophenolic pollutants. The major topics in the biodegradation of these pollutants are focused on the use of specific cultures, co-metabolic degradation and novel biological processes such as biomass immobilization or granular activated sludge. Conversely, only a few studies report the use of mixed cultures and the acclimation of biomass to enhance the biodegradation process. Also, the treatment feasibility is most frequently evaluated based on global and specific degradation efficiencies only. Furthermore, ozone-based processes have been shown to degrade chlorophenols and enhance the subsequent biological process. The focus of scientific research on this topic is addressed to the degradation of single pollutants or the enhancement of the oxidation potential

of ozone by using catalysts or UV light. Only a few attempts report experimental results on the chlorophenol degradation in multiple substrate solutions. Also, the need was demonstrated for additional toxicity and/or biodegradability assessments to evaluate intermediate products. Currently, toxicity assessments are mainly focused on the inhibition of specific microorganisms whereas activated sludge contains a differentiated microbial community. Also, the behaviour (or activity) of the biomass, for example by respirometry, was only monitored in a few studies.

Conflicts of interest

There are no conflicts to declare.

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