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The Effects of Chloride Position on the Aerobic Degradation of Chlorobenzoates by *Klebsiella pneumoniae*

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Abstract

The bacterial strain of *Klebsiella pneumoniae* is a non-motile, encapsulated, lactose-fermenting, facultatively anaerobic, and gram-negative rod, which appears as a mucoid lactose fermenter on the MacConkey agar. The present study shows that this bacterium consumed either 2-chlorobenzoate, 3-chlorobenzoate, 3,4-dichlorobenzoate, or 4-chlorobenzoate as a sole carbon source when grown in aerobic pure cultures. A further enhancement in this bacterial uptake of chlorobenzoate was observed when a 0.2 % yeast extract was supplemented to the pure cultures. *Klebsiella pneumonia* was able to cleave 100 % of the 4-chlorobenzoate ring, 89 % of the 3, 4–dichlorobenzoate ring, 84 % of the 3-chlorobenzoate ring, and 70 % of the 2-chloro-benzoate ring, after an incubation time of seventy-two hours. Concomitantly, the aromatic ring degradation was linked with the release of chloride atoms at a rate of $2.62x10^5$ mol/h from 4-chlorobenzoate, $2.3x10^5$ mol/h from 3,4-dichlorobenzoate, $2.11x10^5$ mol/h from 3-chlorobenzoate and $1.91x10^5$ mol/h from 2-chlorobenzoate, respectively. A mixing of *Enterobacter aerogenes* with *Klebsiella pneumoniae* in a consortium culture had inhibitory effects on this biodegradation process. The present data suggest that a complete enzymatic system is potentially present in *Klebsiella pneumonia* to biodegrade chloroaromatic compounds, and this system is more competent to degrade 4-chlorobenzoate than other investigated chlorobenzoates.

Keywords: *Klebsiella pneumoniae*, Chlorobenzoate, Aromatic ring, Biodegradation

1. Introduction

Polychlorinated biphenyls (PCB) are often released into the environment as a result of natural microbial processes involving the degradation of vast amounts of agricultural and industrial chlorinated organic chemicals mainly produced from the herbicide and pesticide wastes (Monferran *et al.*, 2005; Field and Alvarez, 2008; Sunday *et al*., 2008). Since, aerobic bacteria are incapable of catabolizing these chloroaromatic compounds further (Shields 1985), a more extended biodegradation of PCB is usually terminated with the accumulation of chloro-benzoate intermediates (Adriaens 1991). However, due to the irrelative toxicity and high persistence in the environment, the accumulation of contaminated chloro-benzoates may endanger the water supplies and food chains (Adriaens 1991). Hence, the investigations of chlorobenzoates metabolic fate and their microbial biodegradation should be among researchers' top interests to eliminate their environmental pollutions (Wang *et al.*, 2007). The released chlorobenzoates from their environmental sources frequently contain different numbers and positions of chlorine atoms on the aromatic rings (Adebusoye 2008). However, the influence of chloro-substituent position on the subsequent outcome of chlorobenzoate bio-removal from the environment is not very clear and its exploration may add further knowledge to the understanding of this biodegradation process (Praveena 2007). Similar to other chlorinated aromatic compounds, the chlorobenzoates are relatively stable molecules due to the presence of carbon-chlorine bonds, which tend to hamper this biodegradation process (Hernandez *et al*., 1991). Despite these restrictions, several bacterial strains have managed to degrade chlorobenzoates by adopting certain aerobic and anaerobic metabolic pathways. The aerobic mechanism generally proceeds through the modified ortho-cleavage pathway using chlorocatechols as central intermediates, (Kasberg, 1995) or by hydrolytic dehalogenation with the hydroxybenzoic acid as an intermediate (Radice 2007). So far, very little knowledge is available on the aerobic biodegradation of the chlorobenzoate compounds by the gram-negative bacteria *Klebsiella pneumoniae.* The potential of this bacterial strain to carry out the aerobic degradation of differently chloro-substituted benzoates is investigated in present work.

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2. Materials and Methods

Klebsiella pneumonia and *Enterobacter aerogenes* strains were maintained on a Luria Broth (LB) medium containing 10g Trypton, 10g Sodium chloride and 5g Yeast extract, per one liter.

The bacterial growth on chlorobenzoate compounds as a sole source of carbon and energy was carried out using Minimal Salts Medium (MSM). This medium contained (per one liter) a mixed solution of (1.36 g) KH_2PO_4, (2.43 g) Na_2HPO_2, (0.5 g) $(NH4)_2SO_4$, (0.2 g) $MgSO_4.7H_2O$, (0.002 g) $CaSO_4\cdot$, (0.005 g) $FeSO_4\cdot 7H_2O$, (0.0025 g) $NaMoO_2\cdot 2H_2O$, and (0.0025 g) $MnSO_4$. Each culture was prepared in 50mL of MSM and was supplied with a chlorobenzoate compound. The cultures were inoculated with bacterial cells equivalent to 0.25 OD at 600nm (approximately $5X10^7$ cells/mL), and the growth biomass was checked by determining the absorbance at OD 600 nm. The biodegradation of chlorobenzoate compound was monitored by the release of inorganic chloride, which was estimated turbidimetrically as AgCl precipitation using the wavelength 525 nm (Hickey and Focht, 1990). The levels of chloride atoms were calculated from a standard chloride curve of linear concentration from 0.5 to 2 mM. Additionally, the residual amount of chlorobenzoate remaining after ring cleavage was determined by measuring the decrease in absorbance at 263 nm (Manikandan *et al*., 2007).

The average rate of chlorobenzoate degradation (mM amounts of chloride released or % of aromatic ring cleavage per hour) was estimated from the best fit for the non-linear regression equation. The data of this equation were extrapolated from the initial velocity of chloro-benzoate transformation (approximately the early twenty hours of incubation time) as described previously for the degradation of chloroaromatic phenols (Loh 1998, Mendonça 2004).The initial degradation rate of each compound was calculated by dividing 50 % of the residual aromatic ring remaining or the amounts of chloride released/ the time required to cause this amount of degradation. The reported data represent an average of the values obtained from duplicate experiments.

3. Results

3.1. Optimal Growth Conditions of Klebsiella pneumoniae on Chlorobenzoate Compounds

A preliminary screening of *K. pneumonia* growth conditions on MEM medium resulted in selecting an optimum concentration of chlorobenzoate equal to 3.5 mM at pH 7 and an incubation temperature of 37°C in addition to the agitation rate of 150 rpm (data not shown). Under these optimum conditions, the *K. pneumonia* strain was able to consume 4-chlorobenzoate (4-CBA) as a sole carbon source and attended the stationary phase almost within seventy-two hours, when the bacterial growth was monitored by the OD measurement at 600 nm (Figure 1).

A control culture, incubated for 120 hours without the 4-cholorobenzoate supplement, produced less than one tenth of the total bacterial mass produced in the presence of the chlorobenzoate compound.

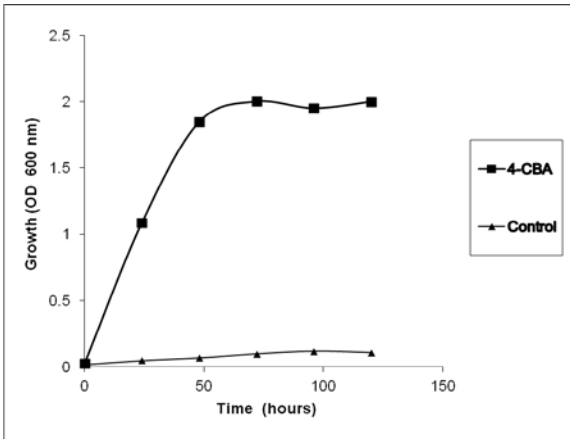


Figure 1. Optimum growth conditions of *K. pneumonia* on MSM. The medium containing 3.5 mM of 4-CBA compound at pH 7, temperature 37°C, and an agitation rate of 150 rpm. Similar culture lacking the 4-CBA was used as a control. Bacterial growth was expressed as OD at 600 nm.

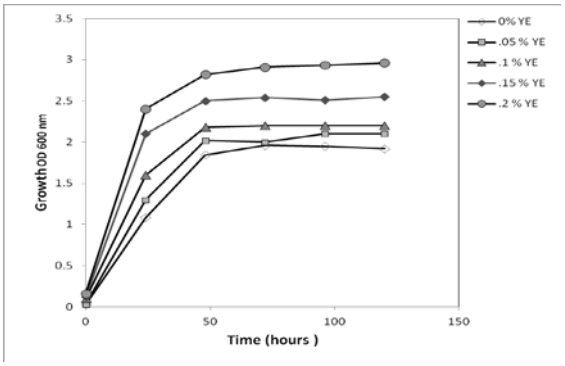


Figure 2. Effect of yeast extract supplementation on the growth of *K. pneumonia* using 4-CBA. The yeast extract was supplemented to the MSM at the following proportions 0, 0.05 %, 0.1 %, 0.15 % and 0.2 %. Growth conditions were similar to those described in the legend of Figure 1. Ye= yeast extract

3.2. Effects of Different Chlorosubstituents on the Growth of Klebsiella pneumoniae

K. pneumoniae was able to grow on either 2-chlorobezoate (2-CBA), 3-chlorobenzoate (3-CBA), 3, 4-dichlorobenzoate (3, 4-CBA) or 4-chlorobenzoate (4-CBA) as a sole carbon source (Figure 3). When the 4-CBA derivative was consumed, this bacterial strain produced a maximum growth of 2.8 OD in addition to attending the stationary phase much quicker than after the utilization of other chlorobenzoates. On the other hand, after the consumption of 2-CBA, this strain showed the lowest bacterial growth (1.5 OD) and the slowest rate to attend the stationary phase. (Figure 3). *K. pneumoniae* expressed a better growth rate on 3-CBA compared to 2-CBA, while after consuming the 3, 4-CBA chloro-substituted compound, an intermediate growth rate between those achieved by the 3-CBA and 4-CBA compounds was obtained.

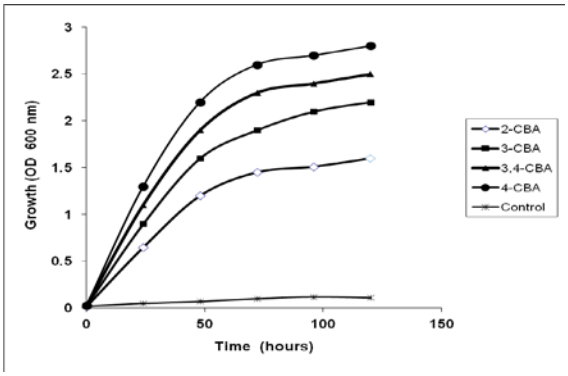


Figure 3. Effects of different chlorobenzoates on *K. pneumonia* growth. The growth of *K. pneumoniae* expressed as OD at 600nm, was measured per time using 3.5 mM of either 2.CBA,3CBA,3,4 CBA and 4-CBA.Conditions were similar to Figure 1,except that a 0.2 % yeast extract was added to the MSM medium.

3.3. Biodegradation of Chlorobenzoates by Klebsiella pneumoniae

All four chlorobenzoate derivatives were degraded by *Klebsiella pneumonia* in the MSM medium, and their efficiency of degradation was expressed in the order 4-CBA →3,4- CB→ 3-CBA→2-CBA.

The process of chlorobenzoate degradation was monitored by two biochemical parameters:

1. Measurement of the residual amount of chlorobenzoate remaining after ring cleavage as determined by the decrease in absorbance of chloro-benzoates ring at 263 nm. The extent of this ring cleavage was estimated in comparison with benzoic acid as a reference. A high rate of ring cleavage was produced by 4-CBA, which was almost equal to the rate of ring breakdown obtained with the benzoic acid (Figure 4). After an incubation time of seventy-two hours both of the 4-CBA and the benzoic acid aromatic compounds exhibited almost 100 % cleavage of their rings by *K. pneumoniae.* Under similar incubation conditions, the other three chlorobenzoates of 2-CBA, 3-CBA and 3,4-CBA showed proportional degradation rates of approximately 70 %, 84 %, and 89 %, respectively.

Astoichiometric extrapolation of the chlorobenzoates biodegradation rate was obtained from the linear portion of degradation curve (table 1). In parallel to the results obtained in Figure 4, the highest rate of ring cleavage by *K. pneumoniae* was obtained with 4-CBA($10.4x10^{-3}$ g/h) followed by 3,4- CB ($9.8x10^{-3}$ g/h) then 3-CBA ($9.1x10^{-3}$ g/h), and the least degradation rate was scored by 2-CBA (8.8×10^{-3}g/h), respectively.

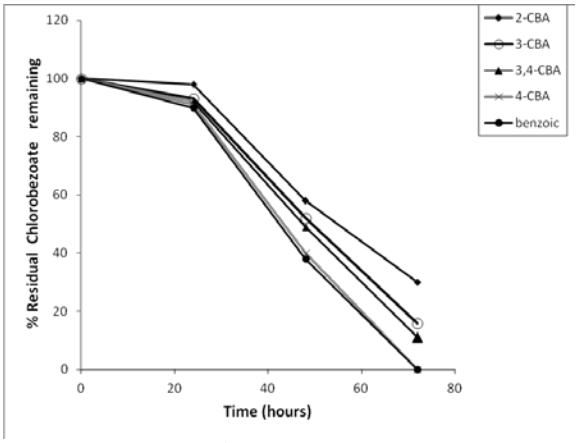


Figure 4. Time course of aromatic ring breakdown. The residual amount of chlorobenzoate remaining after *K. pneumoniae* ring cleavage was determined in the MSM medium, by measuring the decrease in absorbance at 263 nm. Each chlorobenzoate compound was used at the concentration of 3.5 mM.

2. Determining Chloride Release from the Degradation of Chlorobenzoates.

In order to avoid any discrepancy in the estimation of chloride release during the chlorobenzoate degradation, the $CaCl_2$ component in the MSM medium was replaced by CaSO4. Such metal replacement did not mark any significant interference with the rate of *K. pneumonia*e growth on the chlorobenzoate substrates. The bacterial biodegradation of all four chloro-substituted benzoic acid derivatives on this chloride-free MSM medium exhibited different rates of chloride release (Figure.5). The stoichiometry for the rate of this chloride release showed a decrease in the order 4-CBA ($2.62x10^{-5}$ mol/h)>3, 4-CBA ($2.3x10^{-5}$ mol/h) >3-CBA ($2.11x10^{-5}$ mol/h)>2-CBA ($1.91x10^{-5}$ mol/h, respectively (Table 1).

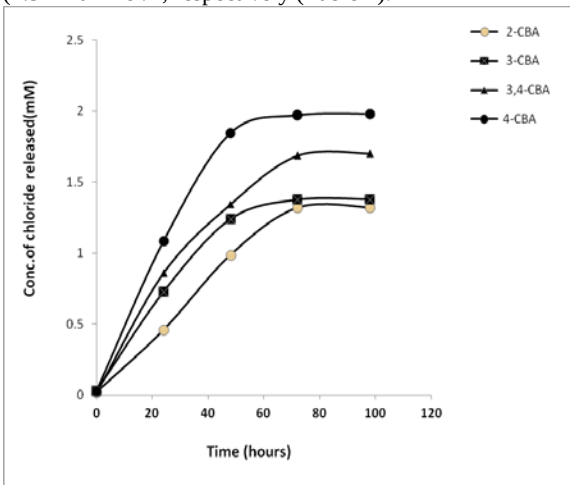


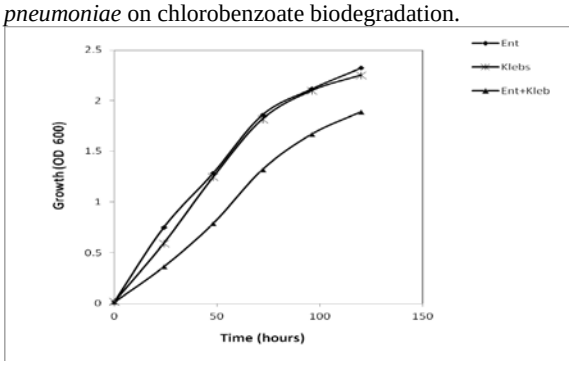
Figure 5. Rate of chloride release. The mM concentration of inorganic chloride release after *K. pneumoniae* degradation was estimated turbidimetrically as AgCl precipitation using the wavelength 525 nm and was plotted against time (hours).

Table 1. Rate of chlorobenzoates' biodegradation. The average rate of each chlorobenzoate (CBA) biodegradation (mM amounts of chloride released or % of aromatic ring cleavage per hour) was estimated from the best fit for the corresponding non-linear regression equation of initial velocity.

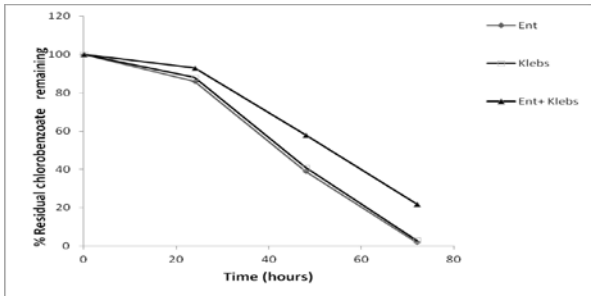
	Type of Chlorobenzoate Derivative			
	2-CBA	3-CBA	3, 4-CBA	4-CBA
Chlorobenzoate ring cleavage (g/h)	8.8×10^{-3}	9.1×10^{-3}	9.8×10^{-3}	10.4×10^{-3}
Chloride released (mol/h)	1.91×10^{-5}	2.11×10^{-5}	2.3×10^{-5}	2.62×10^{-5}

3.4. Comparison of Single Bacterial Species and Mixed Bacterial Consortium to Degrade Chlorobenzoates

A comparison of the bacterial growth curve and the rate of chlorobenzoate biodegradation was conducted between the single culture of *Klebsiella pneumoniae* and a consortium mixture containing this bacterial strain in addition to the gram-negative strain *Enterobacter aerogenes*. Data in Figures 5 and 6 indicate the production of an antagonistic effect by the consortium bacterial mixture that resulted in reducing the actions of *Klebsiella pneumoniae* on chlorobenzoate biodegradation.



(A)



(B)

Figure 6. Comparison of chlorobenzoates biodegradation between single species versus consortium bacteria: (A) Growth curve of 3.5 mM 3-chlorobenzoate in the MSM medium by either the single species of *Enterobacter aerogenes* or *Klebsiella pneumoniae* in comparison to a mixed culture containing both bacterial species. Growth conditions were similar to the legend of Figure 3. (B) Biodegradation of 3.5 mM 3-chlorobenzoate by either the single species of *Enterobacter aerogenes* or *Klebsiella pneumoniae* in comparison to the mixed culture containing both bacterial species. Biodegradation conditions were similar to the legend of Figure 4.

4. Discussion

This study is mainly focused on evaluating the potential of *Klebsiella pneumoniae* to degrade the chlorobenzoate compounds 2-chlorobenzoate, 3-chlorobenzoate, 3, 4-dichlorobenzoate and 4-chlorobenzoate in pure cultures.

The present data showed that all these four chlorobenzoates can be degraded aerobically by this bacterial strain, but the efficiency of degrading the parachloro-substituent is higher than the rest of chlorobenzoate derivatives being investigated. Furthermore, the presence of chloride atoms in the meta-para dichloro-substitution displayed a better degradation efficiency compared to the single chloro-subtitution at the meta- position. An initial lag period of about twenty hours was observed during the degradation of chlorobenzoate rings. This lag period is probably the outcome of a delay in the time required to obtain full activation of the appropriate degrading enzymes. The findings of this study concerning the selective degradation of chlorobenzoates by *Klebsiella pneumoniae* are inferred from the increase in the bacterial biomass, the stoichiometric release of chloride atoms and the proportional amounts of aromatic substrate being cleaved during the degradation process. The researchers conceive that that the bacterial selectively to degrade different chlorobenzoates might be influenced by the potential of these compounds to readily lose the chloride atoms. Naturally, the aerobic bacterial biodegradation pathway of 4-CBA is initiated by an early step of dechlorination to generate the intermediate hydroxybenzoate (Zhuang 2003, Radice 2007). Subsequently, this intermediate undergoes an aromatic ring opening through the β-ketoadipate pathway (Tobita 1992).

On the other hand, in the aerobic biodegradation pathways of 2-chlorobenzoate and 3-chlorobenzoate, the lack of a preliminary step to eliminate chloride atom(s) may force these halogen atoms to remain trapped within the molecular structure of chloroaromatic intermediates (Chatterjee 1981, Hickey 1990, Krooneman 2000, Providenti 2001). A further dechlorination can only take place, when these chloroaromatic intermediates undergo a late ring opening. Thus, a rapid removal of the chloride atom at the early step of the degradation pathway may serve as the driving force that facilitates the biodegradation of 4-CBA.However, in those chlorobenzoates that lack such early dechlorination step, the delay in the elimination of chloride atoms may hamper their degradation efficiency. Noteworthy is when the chloride atom is remained persistently attached to the benzene ring, perhaps interfering with the dioxygenase enzymes action (Scholten1991, Vrchotová 2013, Arora 2014), and leading to an increase in the tendency of chlorobenzoates to resist bacterial biodegradation (Field 2008). This enzymic interference is attributed to both steric and electronic effects, since the chloride atoms have larger and more electron-withdrawing properties than the hydrogen atoms.

In most studied bacterial strains, the biodegradations of chlorobenzoate is mainly affected by the position of the chloro-substituents on the aromatic ring rather than the number of chloride atoms, (Baggi, 2008). However, there is a great diversity among these bacterial strains regarding the effects of chloride substituent position on the selection of chlorobenzoate substrate for biodegradation. Some aerobic bacterial strains are similar to *Klebsiella pneumoniae*, which favor a selective degradation of 4-chloro-substituted benzoates over other types of chlorobenzoates. This category of bacteria includes *Arthrobacter sp.*, (Shimao 1989, Radice 2007, Zhuang 2003,), the *Cupriavidus* sp., (Adebusoye 2017),

Pseudomonas aeruginosa (Hoskeri 2011), *Acinetobacter sp.* (Kobayashi 1998),and *Nocardia* sp. (Klages 1979).

There are few strains in this category including *Acinetobacter sp.* (Kobayashi 1998) and *Arthrobacter sp.* (Vrchotová, 2013) which are so specific towards their biodegradation substrates, and can only degrade 4-CBA, but no other chlorobenzoate substituents.

In contrast, some aerobic strains such as *Rhodococcus erythropolis* strain (Yun 2007) or *Pseudomonas stutzeri* (Kozlovsky 1993) or the bacterial mixture of *Stenotrophomonas maltophilia, Cupriavidus necator* and *Flavobacterium* sp.(Baggi 2008) show high resistance to the catabolism of the para-substituent chlorobenzoate, preferring the degradation of ortho- and/or meta-chlorobenzoates more than the para-chlorobenzoates (Yun 2007).

On the whole, the selective degradation of chlorobenzoates by aerobic pathway seems to depend on the type of bacterial strain, the chlorination position of benzoate compound, the availability of inducible key metabolic reactions, and the presence of a suitable system for uptake.

Data in the current study suggest that a limited enrichment of the *Klebsiella pneumoniae* culture with a yeast extract can improve the bacterial consumption of chlorobenzoates as sole carbon sources. This is agreeable with the reported significance of this nitrogen supplement to enhance the aerobic degradation rate of some xenobiotics (Armenante 1995; Fava 1995).

Although pure cultures can be useful for clarifying certain details on biodegradation pathways, the existence of bacterial strains in community can be environmentally significant in broadening the biodegradative capacity of xenobiotics as well decreasing the burden of toxicity on the biodegradation process (Grady, 1985).

In an attempt to investigate the significance of mixing *Klebsiella pneumoniae* in a consortium culture with *Enterobacter aerogenes* on the rate of chlorobenzoate degradation, this study has found out that such consortium has antagonist effects. Therefore, when both strains are present within a consortium, they show a competition towards the consumption and degradation of 4-chlorobenzoate, which indicates that they share the same degradation pathways of this chlorobenzoate derivative.

5. Conclusion

The *Klebsiella pneumoniae* strain is highly efficient in the degradation of the chlorobenzoate compounds 4-CBA, 3, 4-dCB, 3-CBA, and 2-CBA as carbon and energy sources, but favors the biodegradation of 4-CBA over other chlorobenzoates derivatives. These data highlight the potential of this bacterial strain as a useful candidate to clarify future contaminations of environmental sites with mixtures of Chlorobenzoates, particularly the 4-CBA contamination.

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References

Adebusoye, SA. 2017. Biological degradation of 4-chlorobenzoic acid by a PCB-metabolizing bacterium through a pathway not involving (chloro) catechol. *Biodeg.*, **28**:37-51.

Adebusoye SA, Picardal FW, Ilori MO, Amund OO. 2008. Influence of chlorobenzoic acids on the growth and degradation potentials of PCB-degrading microorganisms. *World J Microbiol Biotechnol.*, **24(7):**1203–1208.

Adriaens P and Focht D D. 1991. Continuous co-culture degradation of selected polychlorinated biphenyl congeners by Acinetobacter spp. in an aerobic reactor system. *J Environ Sci Technol*, **24**: 1042–1049.

Armenante P, Fava F and Kafkewitz D 1995. Effect of yeast extract on growth kinetics during aerobic biodegradation of chlorobenzoic acids. *Biotechnol Bioeng*, **47**:227–233.

Arora P K and Bae H. 2014. Role of dehalogenases in aerobic bacterial degradation of chlorinated aromatic compounds. *Journal Chem.*, 2014: Article ID: 157974.

Baggi, G., Bernasconi S, Zangrossi M, Cavalca L and Andreoni,V. 2008. Co-metabolism of di- and trichlorobenzoates in a 2-chlorobenzoate degrading bacterial culture: Effect of the position and number of halo-substituents. *Inter Biodeter Biodeg.*, **62**: 57-64

Chatterjee DK, Kellogg ST, Hamada S and Chakrabarty AM. 1981. Plasmid specifying total degradation of 3-chlorobenzoate by a modified ortho pathway. *J Bacteriol*., **146(2):**639–646.

C.P.Leslie Grady Jr. 1985. Biodegradation: Its measurement and microbial basis. *Biotechnol Bioeng*., **27**, 660 – 674.

Kozlovsky SA1, Zaitsev GM, Kunc F, Gabriel J, Boronin AM. 1993. Degradation of 2-chlorobenzoic and 2,5-dichlorobenzoic acids in pure culture by *Pseudomonas stutzeri. Folia Microbiol (Praha),* **38(5)**:371-5.

Field J and Sierra-Alvarez R. 2008. Microbial transformation of chlorinated benzoates. *Rev Environl Sci Biotechnol*., **7(3):** 191-210.

Henery S and Grbic-Galic D. 1995. Effect of mineral media on trichloroethylene oxidation by aquifer methanotrophs. *Microb Ecol*, **20**:151–169.

Hernandez BS, Higson FK, Kondrat R and Focht DD. 1991. Metabolism of and inhibition by chlorobenzoates in *Pseudomonas putida* P111. *Appl Environ Microbiol*., **57**: 3361-3366.

Hickey W and Focht D. 1990. Degradation of mono-, di-, and trihalogenated benzoic acids by *Pseudomonas aeruginosa* JB2. *Appl Environ Microbiol*, **56(12**): 3842-3850.

Hoskeri RS, Mulla SI, Shouche YS and Ninnekar HZ. 2011. Biodegradation of 4-chlorobenzoic acid by *Pseudomonas aeruginosa* PA01 NC. *Biodeg*., **22**:509–516.

Kasberg T, Daubaras D L, Chakrabarty A M , Kinzelt D and Reineke W. 1995. Evidence that operons tcb, tfd, and clc encode maleylacetate reductase, the fourth enzyme of the modified ortho pathway. *J Bacteriol*., **177(13):** 3885-3889.

Klages U and Lingens F. 1979. Degradation of 4-chlorobenzoic acid by a *Nocardia* species. *FEMS Microbiol. Lett.* **6**:201- 203.

Kobayashi K, Katayama-Hirayama K and Tobita S. 1998. Metabolic pathway of benzoic acid in an Acinetobacter sp. that mineralizes 4-chlorobenzoic acid. *Jap J Toxicol Environ Health*. **44(1):** 25-33.

Kozlovsky SA1, Zaitsev GM, Kunc F, Gabriel J and Boronin AM. 1993. Degradation of 2-chlorobenzoic and 2,5-dichlorobenzoic acids in soil columns *by Pseudomonas stutzeri*. *Folia Microbiol (Praha)*, **38(5**):376-378.

Krooneman J, Slickers AO, Pedro Gomes TM, Forney LJ and Gottschal JC. 2000. Characterization of 3-chlorobenzoate degrading aerobic bacteria isolated under various environmental conditions. *FEMS Microbiol Ecol*., **32(1):**53–59.

Loh KC and Wang S J. 1998. Enhancement of biodegradation of phenol and a non-growth substrate 4- chlorophenol by medium augmentation with conventional carbon sources. *Biodeg*., **8**: 329-33.8.

Manikandan R, Prabhu HJ and Sivashanmugam P. 2007. Biodegradation of chlorobenzoate using immobilized crude extracts in packed bed column. *African J Biotechnol.,* **6 (19):** 2259-2266.

Mendonça E, Martins A and Anselmo A M. 2004. Biodegradation of natural phenolic compounds as single and mixed substrates by *Fusarium flocciferum*. *Electronic J Biotechnol.*, **7:** 30-37.

Monferran VM, Echenique JR and Wunderlin D A. 2005. Degradation of chlorobenzenes by a strain of *Acidovorax avenae* isolated from a polluted aquifer. *Chemosphere*, **61:** 98–106.

Praveena B, Suresh Kumar M, Sandeep M and Tapan C. 2007. Biodegradation of chlorinated compounds—A Review. *Crit Rev Environ Sci Technol*., **37**:165-198.

Providenti MA and Wyndham RC. 2001. Identification and functional characterization of CbaR, a MarR-6 like modulator of the cbaABC-encoded chlorobenzoate catabolism pathway. *Appl Environ Microbiol*., **67(8):**3530–3541.

Radice F, Orlandi V, Massa V, Battini V, Bertoni G , Reineke W and Barbieri P. 2007. Cloning of the Arthrobacter sp. FG1 dehalogenase genes and construction of hybrid pathways in *Pseudomonas putida* strains. *Appl Microbiol Biotechnol.,* **75(5):** 1111-1118.

Shields M S, Hooper S W and Sayler G S. 1985. Plasmid mediated mineralization of 4-chlorobiphenyl. *J Bacteriol*., **163**: 882-889

Scholten JD, Chang K-H, Babbitt PC, Charest H, Sylvestre M and Dunaway-Mariano D. 1991. Novel enzymic hydrolytic dehalogenation of a chlorinated aromatic. *Science*, **253 (5016**): 182–185.

Sunday AA, Flynn WP, Matthew O I and Olukayode O A., 2008. Influence of chlorobenzoic acids on the growth and degradation potentials of PCB-degrading microorganisms. *World J. Microbiol. Biotechnol.*, **24**: 1203–1208.

Shimao M, Onishi S, Mizumori S, Kato N and Sakazawa C**.** 1989. Degradation of 4-Chlorobenzoate by Facultatively Alkalophilic Arthrobacter sp. Strain SB8. *Appl Environ Microbiol*., **55(2):**478-82.

Tobita S and Iyobe S. 1992. Total degradation of 4-chlorobenzoic acid by an *Acinetobacter* sp. *Water Sci Technol*., **25 (11):** 411-418.

Vrchotová B, Lovecká P, DraDková M, Macková M and Macek T. 2013. Influence of root exudates on the bacterial degradation of chlorobenzoic acids. *Sci World J*, Article ID 872026, 8 pages.

Zhuang Z H, Gartemann K H , Eichenlaub R and Dunaway-Mariano D. 2003. Characterization of the 4-hydroxybenzoyl-coenzyme A thioesterase from *Arthrobacter sp.* strain SU. *Appl Environ Microbiol*., **69(5):** 2707-2711.

Wang F, Grundmann S, Schmid M, Dorfler U, Roherer S, Munch J C , Hartmann A, Jiang X and Schroll R. 2007. Isolation and characterization of 1,2,4-trichlorobenzene mineralizing *Bordetella sp*. and its bioremediation potential in soil. *Chemosphere*, **67**: 896–902.

Yun QI, Lin Z, Z. Olusheyi Ojekunle Z and Xin TAN. 2007. Isolation and preliminary characterization of a 3-chlorobenzoate degrading bacteria*. J Environ Sci*., **19**:332-337.