KINETICS OF CARBOFURAN BIOTRANSFORMATION BY *BACILLUS SP.* AND A PROPOSED HYDROLYTIC MECHANISM

Daniel O. Onunga[1], Anastasiah N. Ngigi[2*] and Isaac O. K'owino[1]

[1]Department of Pure and Applied Chemistry, Masinde Muliro University of Science and Technology, P.O Box 190-50100, Kakamega, Kenya.
[2]Department of Chemistry, Multimedia University of Kenya, P. O. Box 15653-00503, Nairobi, Kenya.

ABSTRACT

Microbial degradation is one of the most efficient and environmental friendly method for the removal of carbofuran and other xenobiotics from contaminated soils. *Bacillus* species previously isolated from the soils of Nzoia River Basin, Kenya, was used to study the kinetics of carbofuran biotransformation to carbofuran phenol at different concentrations in liquid cultures. HPLC and GC-MS methods were used for compound residual analysis. Carbofuran biodegradation best fitted the first order kinetics rate model, with rate constants of 0.7054, 0.6173 and 0.5140 day^{-1} for 50, 75 and 100 $\mu g\ mL^{-1}$ initial carbofuran concentrations, respectively, and a good correlation coefficient of above 0.9. The study reports a new peak for carbofuran phenol at m/z 51 as a result of loss of ethyne from phenyl cation m/z 77 that has not been reported before and also proposes a biotransformation pathway mechanism for the hydrolysis of carbofuran to carbofuran phenol by *Bacillus* sp.
Key words: Carbofuran, *Bacillus* sp., biotransformation, kinetics, hydrolysis, mechanism

INTRODUCTION

Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-ylmethylcarbamate), is a widely used insecticide and nematicide on soybeans, rice, potatoes, fruit and vegetable crops. The use of carbofuran has become of environmental concern not only due to its high rate of use but also because it is toxic, carcinogenic and recalcitrant (Chang et al. 2011). The environmental concern of carbofuran in soils has prompted several studies on biodegradation of the chemical over the years.

Bacteria in soil are known to degrade N-methyl carbamates, particularly carbofuran, via hydrolysis or oxidation (Chapalmadugu and Chaudhry 1993). The hydrolytic degradation produces metabolites that are less toxic as compared to the oxidative pathway (Chaudhry et al. 2002, Rozo et al. 2013). Hydrolysis of carbofuran produces carbofuran phenol which is an initial, major and a less toxic metabolite and methylamine as the degradation products (Topp et al. 1993, Peng et al. 2008).

Methylamine is used as carbon and/or nitrogen source by different groups of bacteria that hydrolyze carbofuran without degrading the aromatic ring (Trabue et al. 2001), although in some cases the aromatic moiety is metabolized (Feng et al. 1997, Yan et al. 2007). Other biodegraded metabolites beyond carbofuran phenol include; 2-hydroxy-3-(3-methylpropan-2-ol) benzene-N-methylcarbamate, 3-hydroxycarbofuran, 4-hydroxycarbofuran, 5-hydroxy-carbofuran and 2-hydroxy-3-(3-methylpropan-2-ol) phenol (Ramanand et al. 1988, Chaudhry et al. 2002, Kim et al. 2004, Yan et al. 2007).

Biodegradation pathway leading to hydrolysis of carbofuran to carbofuran phenol has been proposed (Chaudhry and Ali 1988, Chapalmadugu and Chaudhry 1992). However, the chemistry of the intermediates leading to the formation of carbofuran phenol, methylamine and carbon dioxide has not been reported. Additionally, the hydrolytic biodegradation mechanism of carbofuran to carbofuran phenol has neither been proposed nor reported.

Bacillus species capable of degrading carbofuran was isolated from the soils of Nzoia River Basin (Onunga et al. 2015). The strain was shown to hydrolyze carbofuran to carbofuran phenol. In this study biodegradation kinetics of *Bacillus* sp. using different initial carbofuran concentrations in mineral salt medium (MSM) was investigated. Additionally, the chemical structures of carbofuran and carbofuran phenol were confirmed by GC-MS and hydrolytic pathway mechanism proposed.

MATERIALS AND METHODS

Reagents

Carbofuran (purity 98.5%) and Carbofuran Phenol (purity 99.0%) pesticide standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), HPLC grade methanol and analytical grade chloroform from Sigma Aldrich (Augsburg, Germany through Kobian Limited, Kenya) and Nylon 66 Microfilter (0.45µm) from Kobian Limited (Kenya). For HPLC calibration, individual pesticide standard stock solutions of 100 µg mL^{-1} for carbofuran and 50 µg mL^{-1} for carbofuran phenol were prepared in LC-grade methanol. Standard solutions were appropriately prepared by diluting the stock solution with LC-grade methanol. Serial dilutions of concentrations ranging from 0 to 100 µg mL^{-1} and 0 to 50 µg mL^{-1} were used for the preparation of calibration curves for quantification of residual carbofuran and its biodegradation product, carbofuran phenol, respectively. MSM was constituted in distilled water as follows (in g L^{-1}, pH 7.2): K$_2$HPO$_4$, 3.75; KH$_2$PO$_4$, 1.0; NaCl, 0.25; MgSO$_4$.7H$_2$O, 0.1; CaCl$_2$.H$_2$O, 0.01; 10 mL of trace elements containing 10 mg of Na$_2$MoO$_4$.2H$_2$O, 25mg of H$_3$BO$_3$, 15 mg of ZnCl$_2$, 5 mg of CuCl$_2$, and 10 mg of FeCl$_3$ per liter. The pH was then adjusted to 7.2 using sodium hydroxide solution.

Determination of the effects of initial carbofuran concentration on its biodegradation

Bacillus sp. which had been previously isolated from Nzoia River Basin, Kenya and identified (Onunga et al. 2015), was used for inoculations. 200 µL of Bacillus sp. isolate (of approximately 0.36 OD$_{600}$ MSM) was inoculated into triplicate flasks containing 100 mL of the MSM supplemented with 50, 75, and two sets of 100 µg mL^{-1} of standard carbofuran respectively. The inoculums were then incubated at 32°C in a Wisecube rotational shaker incubator at 150 rpm for 10 days. 10 mL aliquots of the inoculum containing cells and medium were sampled periodically at day 0, 2, 6, 8, 10 for carbofuran concentrations of 50, 75 and one set of 100 µg mL^{-1}. The aliquots were centrifuged at 5000 rpm for 10 min at 25°C. Carbofuran and its metabolites were then extracted from the supernatants three times with 3 mL of chloroform by vigorously shaking for 5 min. The organic extracts were combined, dried over anhydrous sodium sulfate, and evaporated at 40°C by a vacuum rotary evaporator. The extracts were re-dissolved in 1 mL of LC-grade methanol and filtered through nylon membrane filters (0.45µm) for HPLC analysis of residual carbofuran and its metabolites. The other set of liquid culture inoculated in 100 mL of MSM supplemented with 100 µg mL^{-1} carbofuran was wholly extracted as in the above procedure, keeping the ratios, once on day 10 of incubation and subjected to Gas chromatography – Mass spectrometry (GC – MS) to confirm the chemical structures of the compounds.

Analysis of carbofuran and metabolites

The method by Peng et al. (2008) was used for HPLC analysis performed under the following chromatographic conditions: Shimadzu LC-20AT prominence Liquid Chromatograph equipped with SPD-20A Shimadzu prominence UV/Visible detector and phenomenex 00G-4420-E0 (250 × 4.60 mm, 5 micron, HyperClone 5u BDS C-18 130A) column. The mobile phase was methanol-water (1:1) at a flow rate of 0.7 mL min^{-1} at a detector wavelength of 280 nm and an injection volume of 20 µL. The mobile phase was degassed for 15 min before connecting to the pump (LC-10AT VP-Shimadzu A). The experiments were done in triplicates. The retention times of carbofuran and its metabolite, carbofuran phenol, were established by use of reference standards.

GC-MS Analysis

HPLC sample eluents for the metabolite at retention time 15.3 min were collected between 14 to 17 min (after several injections of 20 µL aliquots of extracts from 100 µg mL^{-1} carbofuran concentration variant incubated for 10 days). The eluents were dried over anhydrous sodium sulfate and evaporated at 40°C by a vacuum rotary evaporator. The residues were re-dissolved in 1 mL of LC-grade methanol and filtrated through nylon membrane filters (0.45 µm) and then subjected to GC - MS analysis to confirm the chemical structure of the metabolite. Also, samples extracts from liquid cultures incubated in 100 µg mL^{-1} carbofuran and sampled at day 10 of incubation were analysed by GC-MS after concentration and filtration as described above.

The method of Yan et al. (2007) was used for GC/MS analysis. Analysis was done with a Konic HRGC 4000b gas chromatograph coupled to a KONIK MSQ 12 Mass Detector. 1µL of each sample was injected into the splitless mode in a TechnoKroma TRB5 (Cross-linked 5% Phenyl-95% Methyl Siloxane) capillary column (305m × 0.25mm i.d × 0.25µm film thickness). The injection port temperature was maintained at 200ºC, while the oven temperature was programmed to rise from 70°C to 280°C at a rate of 20°C/min with a solvent delay time of 60 seconds. Helium was used as the carrier gas at flow rate of 1 mL min^{-1}. Mass spectra were recorded in the Electron Ionization (EI) mode

at 70 electron volts scanning the 50-500 m/z range with a scan time of 1300mS and a dwell time of 1912μS, the ion source and transfer line temperature were maintained at 200°C and 250°C, respectively.

Biodegradation kinetics

Residual carbofuran in the liquid cultures quantified by HPLC were used for kinetic studies. The degradation rate constant (k) was determined using the algorithm $C_t = C_o e^{-kt}$, where C_o is the amount of the initial carbofuran (μg mL^{-1}) in MSM at time zero, C_t is the amount of residual carbofuran (μg mL^{-1}) at time t, rate constant, k (μg mL^{-1} day^{-1}) and t (days) is the degradation period (Bano and Musarrat 2004). Ln [C]$_t$ was plotted against time and a straight line graph obtained, with k as the slope of the graph (Choudhary et al. 2006). The carbofuran removal rate from the MSM environment by the isolate was also expressed as the agent's half-life t$_{1/2}$. The t$_{1/2}$ was calculated from the equation ln 2 = -kt. In this study, the amount of carbofuran and metabolite remaining in liquid cultures were measured. The rest of the portion relative to the applied amount is hereby termed as the degraded amount.

RESULTS AND DISCUSSION

HPLC detection of carbofuran and carbofuran phenol

Carbofuran was detected at a retention time of 12.5 min and carbofuran phenol at 15.3 min (Figure 1). Chromatogram peaks that matched the respective retention times of carbofuran and carbofuran phenol standards peaks from the liquid culture extracts were therefore attributed to carbofuran and carbofuran phenol, respectively.
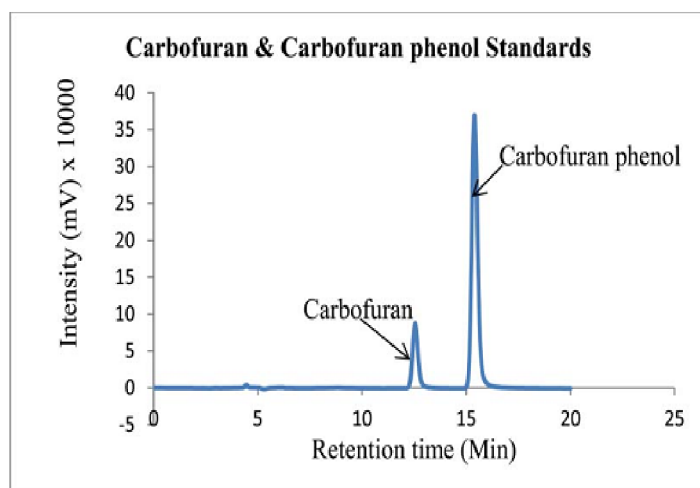

Figure 1. HPLC chromatogram of a standard mixture of carbofuran (R$_t$ = 12.5 min) and carbofuran phenol (R$_t$ = 15.3 min)

Effects of initial concentration of carbofuran on the biodegradation of carbofuran and kinetics of carbofuran degradation

The degradation kinetics of carbofuran by *Bacillus* sp. incubated in MSM with 50, 75 and 100 μg mL^{-1} initial carbofuran concentrations was determined. Linear regressions were obtained as a plot of residual carbofuran against time. The degradation kinetics of carbofuran in MSM with different initial carbofuran concentrations data fitted first-order kinetics. The half-lives (t$_{1/2}$), correlation coefficient R$_1^2$ and carbofuran phenol and residual carbofuran concentrations correlations coefficient, R$_2^2$ are summarized in Table 1.

The *Bacillus* sp. strain showed a higher degradation rate (k =0.7054) for carbofuran at initial concentration of 50 μg mL^{-1} (Table 1). The percent removal was 47 % within the first two days of incubation for the variants with 50 and 75 μg mL^{-1} initial carbofuran concentrations (Table 2). However, degradation was similar, 79 – 99 %, for the three different initial carbofuran concentrations by the 8th to 10th day of incubation (Table 2). Carbofuran was rapidly degraded in MSM with half-lives of 0.9824, 1.2557 and 1.3483 days for 50, 75 and 100 μg mL^{-1}, respectively. Results show that among the concentrations tested, 50 μg mL^{-1} was the optimal concentration with a degradation rate of 0.7054 and a half-life of 0.9824 days. The observed half-life implies an efficient transformation of carbofuran by the isolate at low concentrations. Therefore, *Bacillus* sp. could be considered suitable for degrading carbofuran at low concentrations. The correlation coefficients obtained, R$_1^2$ > 0.9, in all the three different initial carbofuran concentrations showed good linearity between the natural logarithm of the concentrations (Ln[C]) and time, t (days) indicating a good fit for the data to be used for the rates (Plangklang and Reungsang 2012). Since carbofuran phenol was the only metabolite detected, correlations between the residual carbofuran and carbofuran phenol in all the different concentrations gave R$_2^2$ ranging from 0.86 to 0.98 (Table 1).

Structural characterization

Compounds identification in the samples was accomplished by matching the retention times and subsequent structural confirmation of mass fragmentation pattern between the sample and standards of carbofuran and carbofuran phenol. Further, the compounds were identified through library matches using the Automatic Mass Spectral Deconvolution and Identification System (AMDIS) and comparison of the

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Table1. First-order kinetic data for the degradation of carbofuran at various initial concentrations

Initial Concentration ($\mu g\ mL^{-1}$)	Rate constant (k) $\mu g\ mL^{-1}\ day^{-1}$	Half-life (days)	Correlation Coefficient (R_1^2)	Carbofuran phenol and Carbofuran Correlation (R_2^2)
50	0.7054	0.9824	0.9663	0.86
75	0.5519	1.2557	0.9360	0.98
100	0.5140	1.3483	0.9104	0.93

estimated theoretical linear retention index and the calculated linear retention index as per the analysis done. The GC-MS analysis was done on the samples to confirm with certainty the HPLC analysis of the liquid extracts. Also, it was to aid in identifying the presence of any other compounds that may not have been identified through the HPLC analysis. The GC–MS confirmed carbofuran phenol as the only metabolite, as earlier identified by HPLC.

From the GC chromatogram of the standard mixture, carbofuran eluted at about 7.92 min, while the metabolite, identified as carbofuran phenol, eluted at about 4.4 min (Figure 2a). Elution at about 1.05 min was attributed to a solvent peak. GC analysis of liquid culture extracts of *Bacillus* sp. which had been incubated in 100 $\mu g\ mL^{-1}$ carbofuran for ten days as the sole carbon and nitrogen source revealed the presence of one metabolite (Figure 2b). With reference to Figure 2b, the results indicated that all the carbofuran had been degraded within ten days leaving no carbofuran peak in the chromatogram. The only peak present was that of carbofuran phenol at retention time 4.4. The assignments of the chromatographic peaks and retention times were based on a comparison to those of the standard compounds (Figure 2a). The sample eluents extracts which had been collected from the HPLC between retention times 14 – 17 min were

also subjected to the GC analysis. The GC analysis confirmed the presence of carbofuran phenol in the HPLC eluents at $T_{14} - T_{17}$ min (Figure 2c). The peaks in the chromatogram which appeared after that of carbofuran phenol were checked with their mass spectra and found not related to the sample. These peaks may have been caused by the presence of artifacts not associated to the samples.
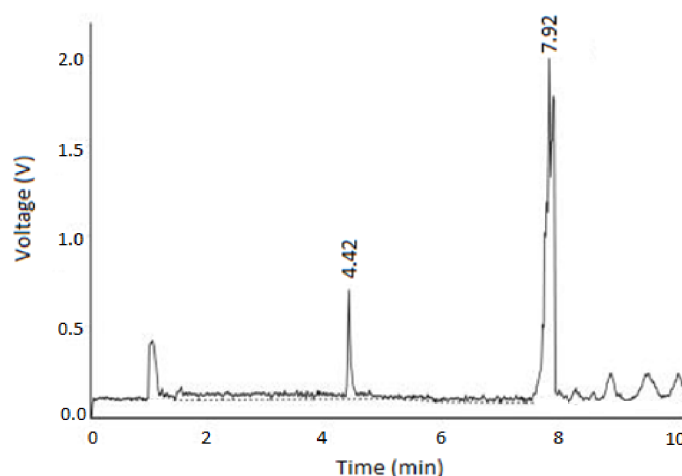
2a

Table 2. The percentage (%) removal of carbofuran by *Bacillus* sp.

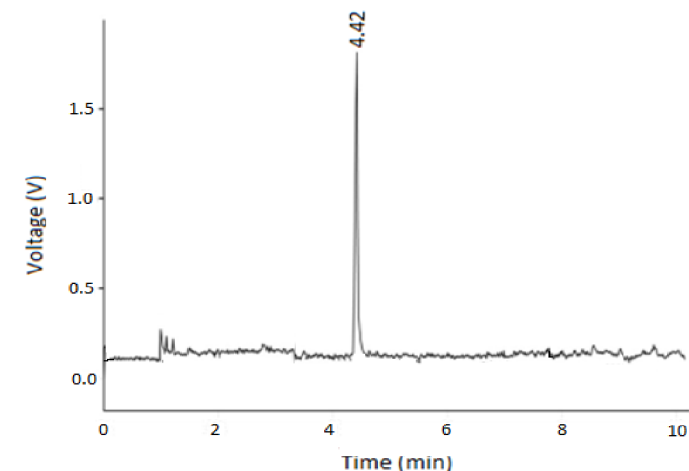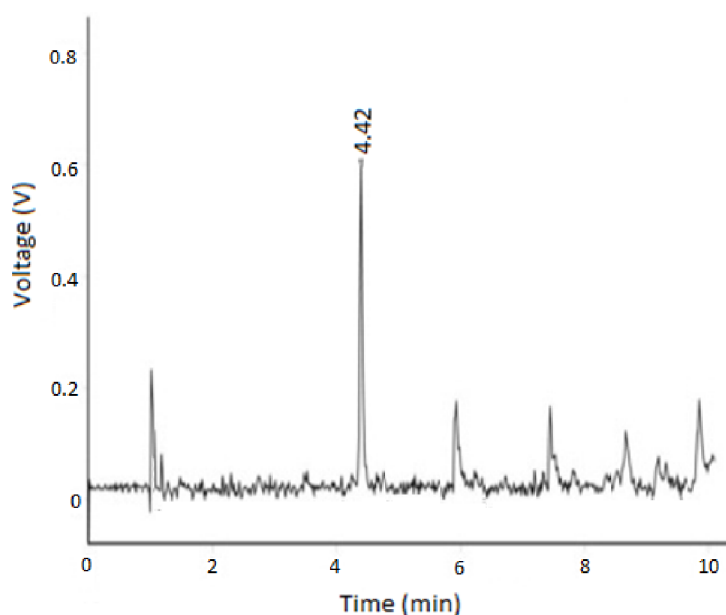| Initial Conc. ($\mu g\ mL^{-1}$) \ Days	0	2	6	8	10
50	0	47.92 ± 3.45	98.32 ± 1.06	99.52 ± 0.95	99.88 ± 0.61
75	0	47.13 ± 5.95	95.35 ± 3.74	98.81 ± 2.62	98.12 ± 0.26
100	0	35.71 ± 9.78	82.14 ± 10.71	98.93 ± 0.85	99.11 ± 0.77

2b

2c

Figure 2. GC chromatograms of **(a)** carbofuran and carbofuran phenol standard mixture, **(b)** the extracts from *Bacillus* sp. liquid cultures supplemented with 100 μg mL⁻¹ carbofuran and incubated for 10 days and **(c)** the eluents collected from the HPLC at the retention times between 14 and 17 min.

Carbofuran had a minor peak at m/z 221 associated with the molecular ion. Other major peaks were at m/z 164 (most abundant), 149, 131, 123, 103, 91, 77, 57 and 51 (Figure 3a). On the other hand carbofuran phenol had major peaks at m/z 164 (molecular ion and most abundant peak), 149,

131, 123, 103, 91, 77 and 51 (Figure 3b). Most of these peaks observed in the mass spectra of carbofuran and carbofuran phenol have been attributed to these compounds by other researchers (Topp et al. 1993, Bachman and Patterson 1999, Chaudhry et al. 2002, Wang et al. 2006). As observed in the spectra, all the peaks of carbofuran and carbofuran phenol were the same except m/z 221 and m/z 57 found only in carbofuran. This difference is as a result of molecular mass of carbofuran (m/z 221) and the 57 mass units' difference as a result of N-Methylcarbamate moeity between carbofuran and carbofuran phenol (Soler et al. 2006, Yang et al. 2013).
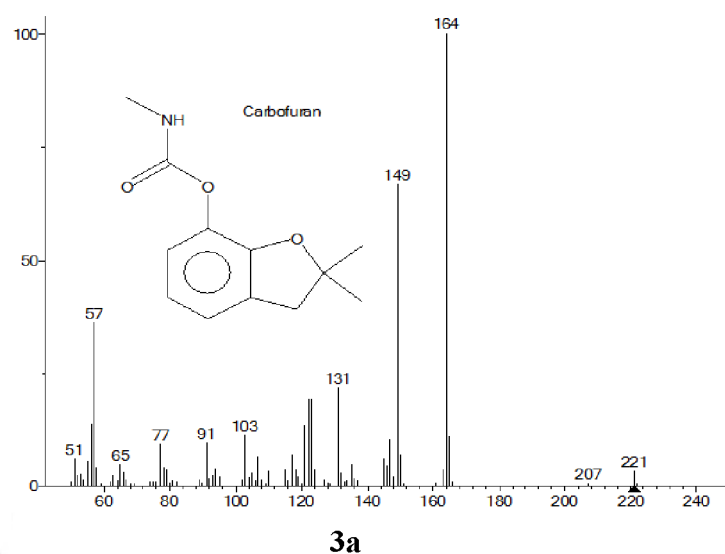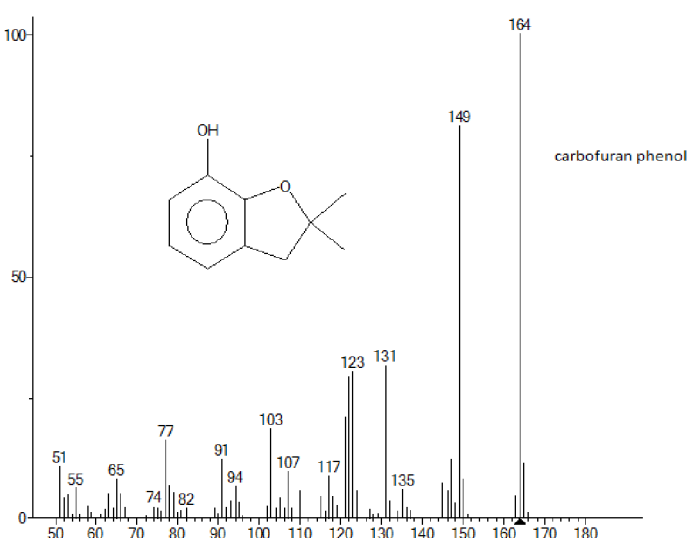

3a

3b

Figure 3. Mass spectra of (a) carbofuran at retention time 7.92 min (b) carbofuran phenol at retention time 4.4 min.

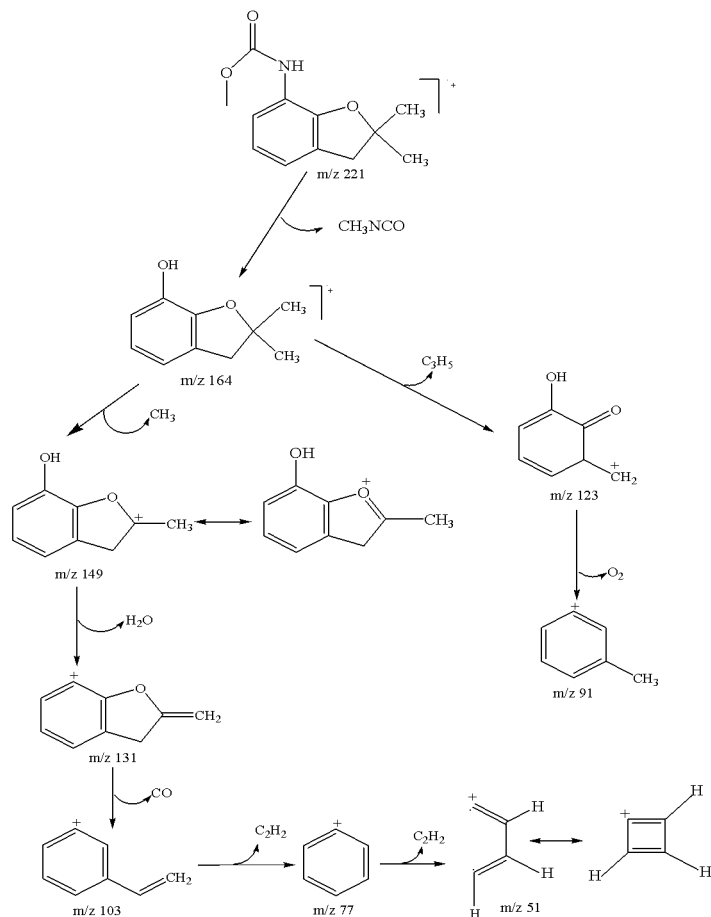## Fragmentation patterns of carbofuran and carbofuran phenol

The mass fragmentation of the metabolite, carbofuran phenol (Figure 3b), had all major peaks similar to carbofuran but 57 mass units smaller for the M^+ peak at m/z 164 than the corresponding mass spectrum of carbofuran with M^+ peak at 221m/z (Figure 3a). The 57 mass units' difference is as a result of the loss of the N-methylcarbamate moiety giving a base peak ion at m/z 164 (Yang et al. 2013, Soler et al. 2006, Hong et al. 1999). The fragment ion at m/z 149 is as a result of the loss of a methyl group from the peak ion at *m/z* 164, as previously reported by Hong et al. (1999) via α-cleavage initiating a charge at the oxygen of the ether linkage by a resonance effect. The fragment ion at m/z 131 was caused by loss of water molecule from the peak ion at m/z 149 (Bachman and Patterson 1999, Kim et al. 2004). On the other hand the fragment ion at m/z 123 was as a result of loss of C_3H_5 from m/z 164 (Hong et al. 1999). The fragment ion at m/z 91, known as tropylium ion, is as a result of the loss of oxygen molecule from m/z 123. Carbon monoxide was lost from fragment ion at m/z 131 to give an ion at m/z 103, while ethyne was lost to give fragment ion at m/z 77 (phenyl cation). The mass peaks were consistent with the findings of other authors who gave the mass fragments of carbofuran and carbofuran phenol as summarized in Table 3.

The mass spectral characteristics of carbofuran phenol in the present study compares to a great extent with the findings of Topp et al. (1993) and Yan et al. (2007) among others with an exception of the ion at m/z 51. Whereas they found same fragmentation characteristics as in this study, a new peak ion at m/z 51 which was not in their work and so far not in the literature of carbofuran and carbofuran phenol spectral characteristics was identified in this study. However, Barker (1999) showed that the phenyl cation ($C_6H_5^+$) ion at m/z 77, from benzyladehyde, is able to undergo homolytic cleavage reaction to give m/z 51 because of the delocalized double bond of $C_6H_5^+$ ion making it possible to eliminate any consecutive carbon atoms (Scheme 1). The electrons in butadiene cation form a bond resulting to cyclobutadiene cation.
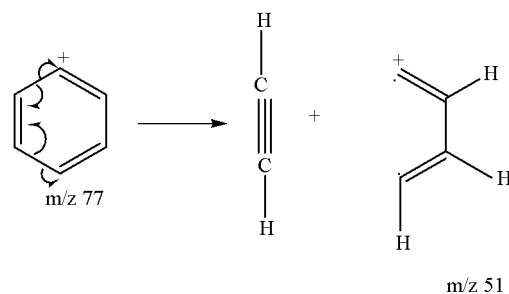
Scheme 1. Fragmentation pattern of phenyl cation (Barker 1999).

Based on the above explanation, this study finds a new ion at m/z 51 as a possible mass spectral characteristic of carbofuran and carbofuran phenol as a result of loss of ethyne from the phenyl cation at m/z 77. The mass spectra peaks assigned confirm carbofuran phenol as one of the degradation products and proposes a fragmentation pattern of carbofuran and carbofuran phenol (Scheme 2).


Scheme 2. Proposed mass fragmentation pattern of carbofuran and carbofuran phenol

Table 3. A summary of mass spectra data of carbofuran and carbofuran phenol

Carbofuran mass spectra	Authors	Carbofuran phenol mass spectra	Authors
221, 164, 149, 131, 122, 103, 91, 77	Chaudhry et al. (2002)	164, 149, 131, 91, 77	Bachman and Patterson (1999)
221, 164, 149, 131, 123, 103, 91, 77	Topp et al. (1993)	164, 149, 131, 123, 103, 91, 77	Topp et al. (1993)
221, 164, 149, 131, 122, 103, 77	Wang et al. (2006)	164, 149, 131, 122, 103, 77	Wang et al. (2006)
221, 164, 149, 131, 123, 103, 91, 77, 57, 51	Present study	164, 149, 131, 123, 103, 91, 77, 51	Present study

Hydrolysis of carbofuran to carbofuran phenol pathway mechanism

From the degradation studies in this work, it can be confirmed that *Bacillus* sp. bio-transformed carbofuran (1) to carbofuran phenol (2), methylamine and carbon dioxide, as methylamine and CO_2 are the other products formed from the hydrolysis of carbofuran. Peaks of both the HPLC and GC chromatograms matched the respective retention times for a standard sample of carbofuran (1) and carbofuran phenol (2). Further, the mass spectra of the products found in the liquid culture extracts were similar to the mass spectra for the standards confirming the identification (Bachman and Patterson 1999), as well as the fragmentation pattern. This *Bacillus* sp. isolate belonged to *Bacillus cereus* or *Bacillus thuringiensis* by identification (Onunga et al. 2015). The carbofuran hydrolysis mechanism by this bacterium is as proposed in Scheme 3. Carbofuran (1) resonates to structure 2 which allows a 1, 3- methyl migration from O to N. This migration is due to the lone pair of electrons in nitrogen giving structure 3. With the aid of decarboxylase enzyme, CO_2 could then be lost to obtain a more stable structure 4. Since N in structure 4 is basic, it can pick a proton from the system to form structure 5 making the amino group on this structure a better leaving group. With the aid of hydrolase enzyme, hydrolysis takes place to form structure 6 after release of methylamine. Since structure 6 is unstable, an enzyme base (Enz-B:) in the system picks a proton to form a stable structure carbofuran phenol 7 as the end product.
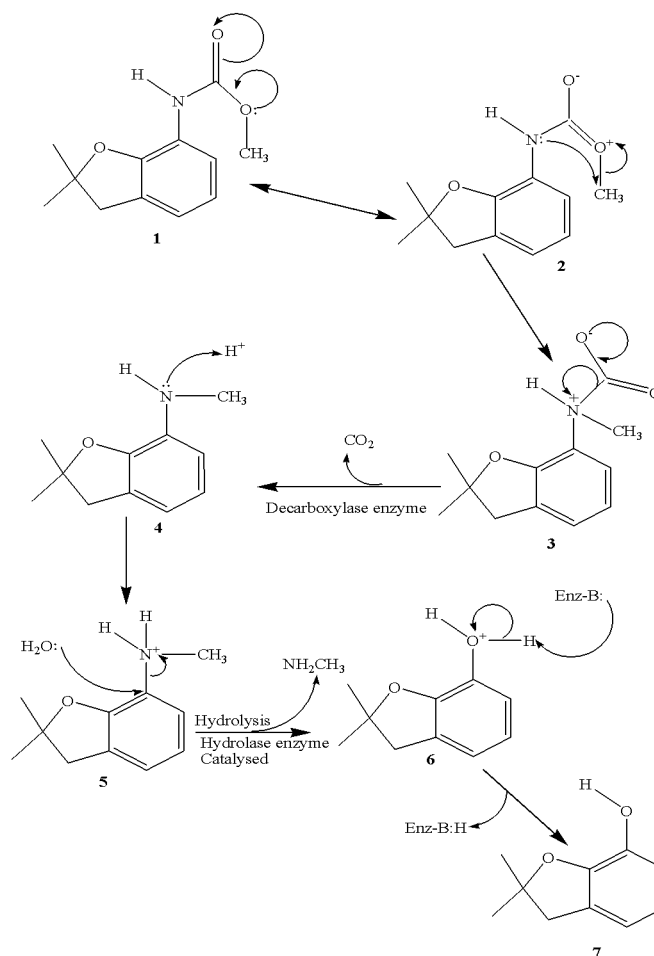
Scheme 3. Proposed mechanism of hydrolysis of carbofuran to carbofuran phenol by *Bacillus* sp.

It is worth noting that bacteria in soil are known to degrade N-methyl carbamate, particularly carbofuran, via hydrolysis or oxidation (Chapalmadugu and Chaudhry 1993). The hydrolytic degradation produces metabolites that are less toxic as compared to the oxidative pathway (Chaudhry et

al. 2002, Rozo et al. 2013). Hydrolysis of carbofuran produces carbofuran phenol which is an initial, major and a less toxic metabolite (Peng et al. 2008), methylamine and carbon dioxide.

Bacillus sp. strain capable of biodegrading carbofuran has been previously reported. For example, Mohapatra and Awasthi (1997) reported biodegradation of applied carbofuran to almost completion after 30 days in a MSM by *B. pumilis*. They reported that the loss of 97% of applied carbofuran, in a mixture of more than two cultures, within 10 days of incubation indicated enhanced degradation of carbofuran by the carbofuran enrichment cultures. In this study *Bacillus* sp. isolate degraded more than 98% of all the different initial carbofuran concentrations applied to carbofuran phenol after 10 days (Table 2) indicating enhanced carbofuran degradation. The enhanced biodegradation of carbofuran by *Bacillus* sp. isolate in the present study may have been caused by long exposure of carbofuran to the soil microorganisms as a result of repeated applications (Mohanta et al. 2012).

The hydrolysis of carbofuran by the isolate *Bacillus* sp. in this study is consistent with previous reports (Chaudhry and Ali 1988, Topp et al. 1993, Peng et al. 2008). They reported bacterial hydrolysis of carbofuran to methylamine, carbon dioxide and carbofuran phenol, as the major product, with other bacterial cultures. Though the biodegradation pathway has been established, the hydrolytic biodegradation mechanism has not been proposed. Methylamine is used as a source of carbon and/or nitrogen by bacteria that hydrolyzes carbofuran without degrading the aromatic ring (Trabue et al. 2001). However, in some cases the aromatic moiety is metabolized (Feng et al. 1997, Yan et al. 2007). *Achrobacter* sp., *Pseudomonas* sp. 50432, *Sphingomonas* sp. strain SB5 and *Novosphingobium* sp. FND-3 have been reported to degrade carbofuran beyond carbofuran phenol (Ramanand et al. 1988, Chaudhry et al. 2002, Kim et al. 2004, Yan et al. 2007). Carbofuran degrading soil bacteria are geo-specific. For example, those from Beijing, China are of *Paracoccus* sp. YM3, while those from Xuzhou, China are of *Novosphingobium* sp. FND-3. Also, those from Florida, USA are of *Pseudomonas* and *Flavobacterium* group, while those from Gwanju, South Korea are of *Sphingomonas* sp. strain SB5, which are all different from those found in other geographical locations. From the soils of Nzoia River Basin in Kenya, particularly Bunyala Rice

Irrigation Scheme, *Bacillus* sp. capable of degrading carbofuran are found.

CONCLUSION

This study showed that *Bacillus* sp. degraded carbofuran to carbofuran phenol in MSM. This degradation followed first order kinetics, with high degradation rate constants (k), for the three (50, 75 and 100 μgmL^{-1}) initial carbofuran concentrations. The isolate *Bacillus* sp. is well adapted for the removal of carbofuran at low concentration as evidenced by high degradation rate. A degradation mechanism of hydrolysis of carbofuran to carbofuran phenol is proposed in this study. This study is helpful for further studies on the mechanism of biodegradation of carbofuran.

ACKNOWLEDGEMENTS

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