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Chlordecone dechlorination under aerobic conditions by *Bacillus* sp

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ABSTRACT: The aerobic dechlorination of chlordecone by bacteria from agricultural organochlorine-used soil of Cameroon South-West region is reported. Selection of microorganism strains having great affinity and/or resistant to chlordecone has been carried out. The effects of physicochemical factors (pH, chlordecone amount, incubation time and temperature) on bacteria growth and the biodegradation of chlordecone were investigated. A *Bacillus* strain has been isolated and was able to resist and grow with chlordecone as sole carbon source. Among physicochemical parameters studied, chlordecone amount had not significant effect on the Bacillus growth in synthetic medium. Free chlorine obtained after incubation of *Bacillus* sp in the presence of 1 µg/mL of chlordecone as sole carbon source showed a maximum released after 10 days, equivalent to dechlorination of 19.5% of total chlorine in the synthetic medium. This report is the first relative to chlordecone dechlorination under aerobic conditions by *Bacillus* strain from African ecosystems.

KEYWORDS: Chlordecone, Dechlorination, *Bacillus sp.*, African ecosystem.

1 INTRODUCTION

Chlordecone (CLD) is a synthetic substance found out in 1951 and used since 1958 as pesticide. Commercially known as Kepone, GC-1189 and Curlone, CLD has been used throughout the world mainly but not exclusively for the control of banana tree weevils in French West Indies, Asia and Africa [1],[2],[3],[4]. Indeed, CLD had been used in Cameroon and Ivory Coast for banana plants preservation [1],[5]. In spite of its high efficiency, CLD shown a toxicity discovered in 1975 in USA involving numerous neurological affections [6],[5]. Its prohibition in France took place in early 1993, but it was used in Cameroon until 1995 and co-used until 2009 in China as impurity of Mirex, another chlorinated pesticide with similar chemical structure. Finally, CLD had been added to Stockholm convention on persistent organic pollutants in 2009 [7].

CLD ($C_{10}Cl_{10}O$) is an organochlorine compound with a peculiar chemical structure and the high steric hindrance caused by the ten chlorine atoms. It belongs to the bishomocubane family characterized by a chemical structure in cage-shape and a high stability [8]. Its low solubility in water gives it a high affinity for organic matter illustrated by a high octane-water partition coefficient (K_{oc}) [2].

To fight against harmful insects, CLD had been applied in soil, in direct contact with plant tree. Due to its chemical structure resistant to chemical reaction and microbial degradations, contamination by CLD can persist several decades to half millennium in natural environment [9]. Pollution can therefore migrate from soil to surface and groundwater by rainfall and leaching. Indeed, CLD had been detected in water up to authorized maximum limit for this pesticide in drinking water (0,1 µg/L) [10] ,[2]. In order to limit human population contamination by this pesticide, drinking water treatment plants in contaminated agricultural zones were equipped for CLD remediation [11], [12]. Many techniques have been used to remediate CLD from water including adsorption on activated carbon (AC), dilution and biological processes [11]–[14] . For CLD removal, AC produced from sugar cane bagasse had been tested [15], [16], [17] ,[11], [12], [18],[12]. However this method of remediation makes a transfer of pollutant from one matter to another and contaminated AC should be regenerated after adsorption processes or confined. Thus, due to their no selectivity property, advanced oxidation processes (AOPs) can be used for carry out total

destruction of CLD. These techniques use hydroxyl radicals which attack organic compounds and can result in complete mineralization of them. Nevertheless, AOPs are expensive due to the cost of materials needed [19]. Another solution can be the microbial degradation of CLD.

Theoretically, numerous properties of CLD like chemical structure in "cage shape", steric congestion due to 10 chlorine atoms, low water solubility and high hydrophobicity suggest that this molecule would be difficult to destroy biologically [20]. However, many authors report biological degradation of CLD [21]. Reference [22] reports transformation of CLD in hydroCLD by-products from aerobic bacteria *Pseudomonas* species isolated from James River (USA) sediments. Results obtained in aerobic conditions shown that total mineralization of CLD was not attained and its cycle structure was safe. Opening of this cycle had been suggested in anaerobic conditions by methanogenic microorganism, *Methanosarcina thermophile,* though mineralization was also not reached [21]. Despite no mineralization of CLD with microorganism already studied, this way have not yet been exhaustive because, all microbial community of various contaminated ecosystem have not been identified yet and studied on CLD remediation [23], [24]. Though microbial consortia isolated from a contaminated site in USA or in the FWI had been used on CLD degradation, few publications are related to African ecosystem. Indeed, CLD had been used in Cameroon and Ivory Coast for banana plants preservation [1], [5]. Therefore, study on microorganism isolated from African ecosystem where CLD had been used can be carried out to know their capability to growth on CLD or use it as carbon source. In the present study, aerobic bacteria extracted from Cameroon banana plantation soil had been tested for CLD degradation.

2 MATERIAL AND METHODS

2.1 CHEMICALS

Analytical standard Chlordecone (PESTANAL®, 99%) was purchased from SIGMA-ALDRICH CHEMIE Gmbh, Riedstrasse, Germany. Lindane (97 %) and Diethyl ether (99.8%) were procured from Sigma-Aldrich. Other chemicals and reagents used in this study were of technical grade and were purchased from chemical companies.

2.2 SOLUTIONS PREPARATION

Stock solution of chlordecone was prepared by dispersed 1.6 mg of chlordecone in 1 liter of demineralized water by moderated ultrasonication (BRANSONIC 221). For the different experiments, an aliquot of stock solution is added aseptically to mineral medium to reach desired chlordecone amount. Mineral medium was prepared by KH_2PO_4 (0,760 $g.L^{-1}$), Na_2HPO_4 (5,455 $g.L^{-1}$) and NH_4NO_3 (0,250 $g.L^{-1}$) in demineralized water.

2.3 SAMPLE COLLECTION

Soil samples were collected in Tiko, Cameroon South West region **(**4°07'00.8"N 9°22'57.0") which had a story of pesticide application. Soil cores (2-20 cm) taken from selected spots were collected in sterilized glass bottles. The samples were iced during transport to laboratory and stored at 4°C until microbial isolation.

2.4 SOIL CHARACTERIZATION

Physicochemical properties of soil were analyzed; typically, textural analysis, moisture content, pH, mineral nitrogen concentration, organic matter, available phosphorus, exchangeable potassium and sodium, sulphate, free chloride and total iron.

Soil texture was evaluated by the determination of two (02) primary particle size fractions. Proportions of sand particles (0.050 mm to 2 mm) and silt and clay (≤ 0.050 mm) have been determined using the NF X 31-107 standard.

Mineral nitrogen assay was performed using a HORIBA LAQUAtwin B-743 selective electrode specific for nitrate ions and, for ammonium ions, by colorimetry at 470 nm using the HANNA HI 733B Checker® colorimeter according to the Nessler method.

Available mineral elements were analyzed after extraction by acetic acid, ammonic acetate and Ethylene diamine tetraacetic acid (EDTA) buffer [25]. Sodium determination was carried out by electrometry (HANNA HI98202) and potassium by electrometry (HORIBA LAQUAtwin B-731) according to the designers' recommendations. The colorimetric technique (HACH 1455-BL) was used for the determination of total iron.

The water-soluble sulphates were assayed by turbidimetry using the modified barium sulphate method (HANNA HI38000). Phosphate and free chlorine concentrations were determined by colorimetry (PRECISION LABS 800-733-0266) and by titration (HACH Quantab® 27449-40), respectively, according to the manufacturers' instructions.

2.5 ISOLATION OF BACTERIAL STRAIN

Bacterial isolation was carried out by the enrichment culture technique using mineral broth (pH 6.8 ±0.5) [26]. Ten grams of collected soil were added to 100 mL of sterile mineral medium containing 10 $mg.L^{-1}$ of lindane. Lindane has been used as organochlorine compound for primary screening before selection with CLD. Erlenmeyer flasks were shaken continuously in a water bath agitator and incubated at 28 ± 2° C for 2 days. Afterwards, 10 mL aliquots from the previous flasks were transferred to a sterile fresh mineral medium containing the same lindane concentration. After 2 transfers, bacteria colonies were isolated by serial dilution and spread plating on Plate Count Agar (PCA). Microorganisms in the final subcultures were isolated on the basis of size, colour and morphology. Purification of cultures was reached by serial subculture on PCA plates.

2.6 SELECTION OF BACTERIA CAPABLE TO GROWTH ON CHLORDECONE

Microbial isolates were screened on agar plates by the method of spray plates [27]. At first, isolates were cultivated on lindane agar plates and preselected strains were tested on chlordecone spray plates. Spray plates were realized containing 1.5% agar in mineral medium and isolated strains were inoculated on the plate. The surface of the pre-settled plates was sprayed with 10% (wt/vol) of lindane in diethyl ether and plates were incubated at 28°C for 7 days. The isolates capable of growing with lindane were selected and incubated on agar plates with chlordecone as source of carbon. An aliquot of the chlordecone stock solution is added aseptically to the mineral medium agar (1.5%) to reach 1.5 $\mu g.mL^{-1}$ chlordecone. The mixture was shaken thorough and poor in Petri dishes. After solidification of the selective medium, pre-selected isolates were inoculated on the surface and incubated at 28°C for 7 days. Bacteria capable to growth on chlordecone selective medium were selected and further characterised.

2.7 CHARACTERIZATION OF THE SELECTED BACTERIUM

Some morphological and biochemical characters of the selected bacterial isolate were analyzed. Microbial enzymatic system was studied during 24 hours by using 12 tests of an API®BioMérieux biochemical system (the first 12 tests diverted from the gallery API20E normally used for Enterobacteria). Metabolites produced during incubation time are translated by color changes due to colored indicator modification with pH changes inside the different media. Also, a specific medium (*Bacillus* cereus Selective Agar PEMBA) was used to discriminate between the kind of the selected bacterial isolate.

2.8 INFLUENCE OF PARAMETERS ON SELECTED BACTERIA GROWTH WITH CHLORDECONE IN CULTURE BROTH

To study the effect of pH, incubation temperature, incubation time and initial chlordecone concentration on growth, a two-level full factorial design was performed. Levels of the factors are shown in table 1. The experiments were realized into Erlenmeyer flasks (100 mL) containing 20 mL of medium. The acclimated bacteria culture was added to the mineral medium containing chlordecone to obtain $2.42.10^8$ CFU/mL. Inoculum was obtained by growing the isolate in Tryptic Soy Broth (TSB, Corning™) containing 100 ppb of chlordecone for 24 h. Thereafter, cells were harvested by centrifugation (6000 g, 30 min at 4°C), washed three times with sterile mineral medium and suspended in required volume of mineral medium for further experiments which were started in 24 h after harvest. Bacteria growth was assessed by indirect measurement of cell numbers by turbidimetric techniques using optical density measuring at 600 nm (OD_{600}) of the inoculated medium after incubation time. The results were statically analyzed using STATGRAPHICS Centurion XV.II (version 15.02.0006, StatPoint, Inc.) and the factors were considered significant when $p < 0.05$.

Table 1. Levels of factors for bacteria isolate growth on chlordecone medium

Factors	Low (-)	High (+)
pH	5	7
Incubation temperature (°C)	23	30
Chlordecone concentration (µg/mL)	0.5	1
Incubation time (hours)	50	100

2.9 DEGRADATION STUDIES

Experiments were conducted according to the modified procedure of [28] and [29]. Assays were performed in 20 mL sterile glass vials containing pre-autoclaved mineral medium at 121°C for 20 min and an aliquot of chlordecone aqueous stock solution. Final chlordecone concentration was 1 µg.mL^{-1} and the inoculum was added to give final cell density which was determined by plate counts on PCA. Initial optical density of the medium was 0.33 AU corresponding to 3.1.10^{2} CFU.mL^{-1}. All the experiments were carried out in triplicate. Flasks, used for inoculum preparation and the experimental flasks were incubated on a shaker water bath (100 rev.min^{-1}) at 28°C. Three flasks were removed at required intervals for analyses such as inorganic chloride and optical density measurements. Blank flask has been primarily inoculated then autoclaved at 121°C for 30 min and maintained as a control [30].

2.10 ESTIMATION OF CHLORDECONE MINERALIZATION

Mineralization of chlordecone by bacterial isolate was inquired by quantifying chloride ion release. 10 mL of cell free supernatant (6000 g, 45 min) from each of the triplicates were removed after 0, 5, 10, 15, 20, 25 and 30 days for the estimation of free chloride following an adaptation of USEPA method 330.5. The reaction between free chlorine and the DPD reagent causes a pink tint in the sample and developed color was measured by a specific photometer (HANNA HI 701) at 525 nm.

3 RESULTS

3.1 SOIL CHARACTERIZATION

The microcosm used for isolation of bacteria degrading organochlorine was analysed and physicochemical properties of the soil sampled is presented in table 2. The soil texture analysis presents 60.97 % of sand and 36.34 % of silt and clay mixture. These soil fractions are representative of "sandy" soil and particularly "silty sand" or "clayey sand" according to the silt or clay contents respectively. Soil samples were acid (pH_{water} 4.51 ± 0.63) and present 13.71% of organic matter, a non-negligible amount. The chemical composition presents a considerable quantity of exchangeable ions, especially important free chlorine and potassium amounts.

Table 2. Physicochemical properties of the agricultural soil sampled

Characteristics	Value
Sand (%)	60.97 ± 1.25
Clay + silt (%)	36.34 ± 0.91
pH_{water}	4.51 ± 0.63
Humidity (%)	14.9 ± 0.82
Organic matter (%)	13.71 ± 0.22
Mineral nitrogen : NO_3^-+NH_4^+ (ppm)	56.32 ± 3.41
Potassium K^+ (ppm)	140 ± 4
Sodium Na^+ (ppm)	21± 1.1
Total iron (ppm)	5 ± 0.4
Sulphate (ppm)	60 ± 3
Phosphate (ppm)	100 ± 5
Free chlorine (ppm)	151 ± 7

3.2 ISOLATION, SCREENING AND CHARACTERIZATION OF BACTERIA ISOLATES

29 bacteria isolates were obtained by enrichment cultures from agricultural soil samples (data not shown). Among these isolates plated on lindane solid medium for preselecting experiments, only 2 isolates shown a visible growth on selective medium (fig.1). After incubation onto chlordecone solid medium, only 01 bacterium was selected and further characterised.

Morphological characterization of selected isolate showed a Gram-positive rod-shaped bacterium (fig. 1). Biochemical analysis had presented catalase positive and oxidase negative responses added to aerobe and facultative anaerobe behaviour of the selected bacteria. Indeed, positive reaction for Arginine and Lysine substrates degradation, which translate presence of Arginine Dihydrolase (ADH) and Lysine Decarboxylase (LDC), could express the facultative anaerobe character of the bacteria (fig. 2). The aerobic breath is presented by positive response for glucose (GLU) character. Finally, bacterium isolate has been

related to *Bacillus sp* on the basis of growing onto *Bacillus* PEMBA agar specific medium (fig. 2). A positive growth is an indication of the presence of the *Bacillus* kind. Indeed, this medium is made selective by presence of antibiotic polymyxine B which is a selective agent for the *Bacillus* kind bacteria. As seen, the selected *Bacillus* species is mannitol positive and lecithinase negative due to yellow color of the colonies and absence of opacity of the medium around them.

Fig. 1. ***Bacterium strain isolated from sampled soil; Left: Bacteria isolate on lindane spray plate; Right: Bacteria isolate under optical microscopy***

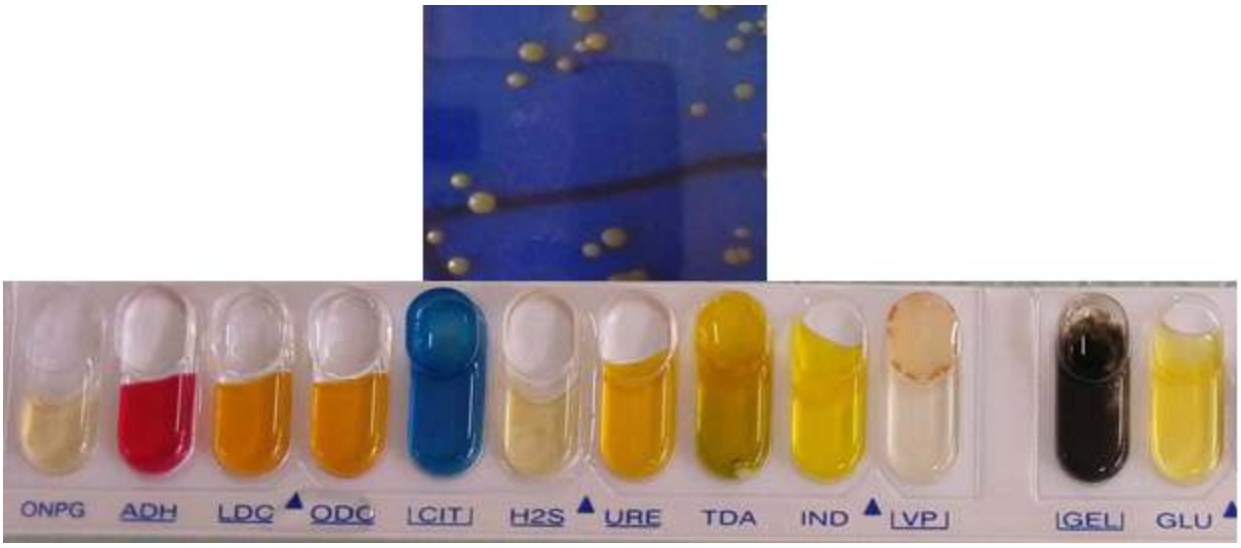

Fig. 2. ***Results of characterization and identification tests; Up: Isolate on Bacillus PEMBA agar; Down: protein metabolism and microbial enzymatic system studies***

3.3 EFFECT OF SOME PHYSICOCHEMICAL PARAMETERS ON *BACILLUS SP* GROWTH

Influence of pH, incubation temperature, incubation time and initial chlordecone concentration on *Bacillus sp* growth was investigated. Results obtained are presented in figure 3 and table 3.

ANOVA table (table 3) presents biomass variability for each separated effects. It checks also the statistical meaning of each effect by comparing quadratic average with regard to an estimation of the experimental error. In our case, 4 effects have probability lower than 0.05, indicating that they are significantly different from zero at the level of 95.0 % confidence. From obtained results, two direct parameters (temperature and pH), and two interactions (temperature – pH and Temperature – time) shown significant effect on *Bacillus sp* growth. Based on these statistical significant effects, the regression equation of model that can predict evolution of the biomass of *Bacillus sp is as follows:*

Biomass of *Bacillus sp* (Optical Density) = 0.06836 - 0.02887*Temperature – 0,04588*pH - 0.02478*Temperature*pH – 0,01888*Temperature*Time

Nevertheless, direct effects of temperature and pH were predominant inside the experimental domain (figure 3). Therefore, acid pH and temperature below 30°C were used for study degradation of CLD in batch reactor.

Table 3. Analysis of Variance for optical density of Bacillus sp growth with chlordecone as carbon source

Source	*Sum of squares*	*DDL*	*Quadratic average*	*F-Ratio*	*Probability*
MAIN EFFECTS					
A:Temperature	0.00333506	1	0.00333506	13.40	0.0064
B:CLD Concentration	0.00004556	1	0.00004556	0.18	0.6800
C:pH	0.00841806	1	0.00841806	33.83	0.0004
D:Time	0.00088506	1	0.00088506	3.56	0.0960
INTERACTIONS					
AB	0.00006006	1	0.00006006	0.24	0.6364
AC (Temperature- pH)	0.00237656	1	0.00237656	9.55	0.0149
AD (Temperature- Time)	0.00142506	1	0.00142506	5.73	0.0436
BC	0.00041006	1	0.00041006	1.65	0.2352
BD	0.00047306	1	0.00047306	1.90	0.2053
CD	0.00049506	1	0.00049506	1.99	0.1961
Residual	0.00199080	8	0.00024885		
Total (corrected)	0.01991440	18			

3.4 *BACILLUS SP.* GROWTH IN CHLORDECONE MEDIUM

Degradation of chlordecone by *Bacillus sp* was investigated. Figures 4, 5 and 6 present the kinetics of bacterial growth, free chlorine released and pH change, respectively. Multiple phases of bacterial growth are observed. Firstly, a decline of biomass until 5 days followed by a microorganism growth. Secondly, a decreased of biomass after 10 days, preceding another increase of bacteria in the medium. After 20 days, a continuous decline of biomass is observed.

Figure 4 shows the evolution of free chlorine released in the medium and blank. It should be noticed that an increase of free chlorine content is monitored before 5 days thought the microbial biomass decreased. Maximum free chlorine released and detected corresponds to only 19.5% of total chlorine expected if all the initial chlordecone had been dechlorinated. Such quantity shows that chlordecone is not completely degraded by *Bacillus* sp.

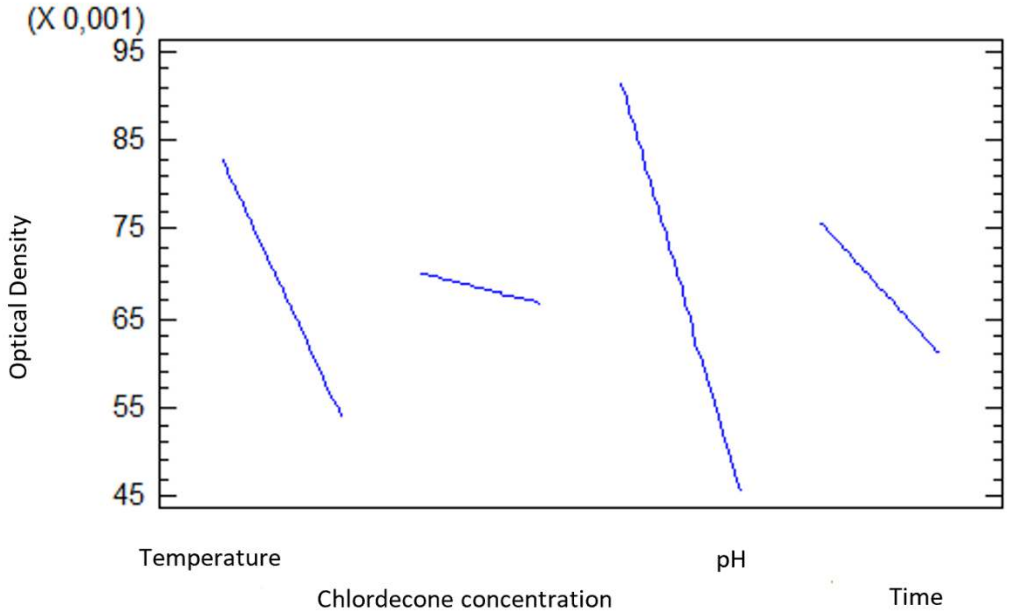

Fig. 3. Graphic of direct effects of studied parameters on Bacillus sp growth

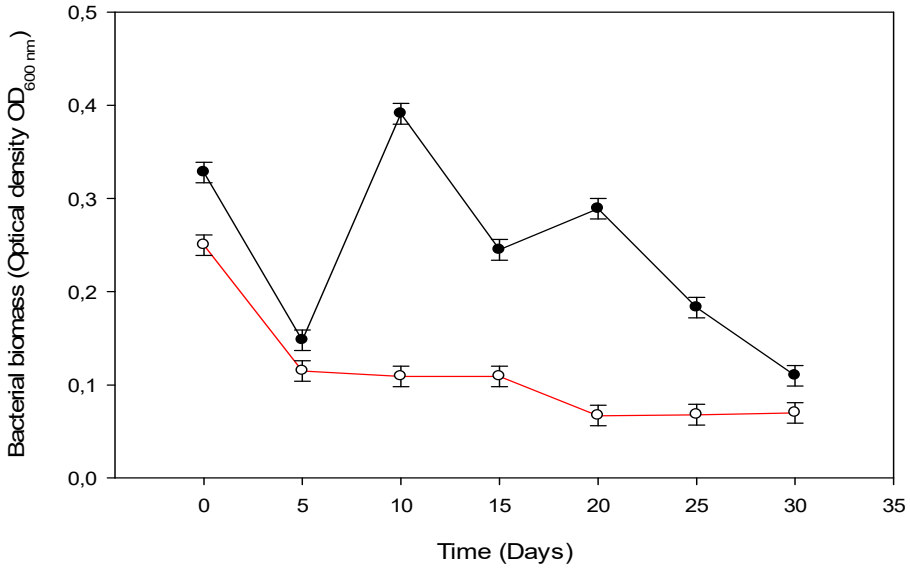

Fig. 4. Kinetic of Bacillus sp growth in liquid medium; T=27±2°C, [chlordecone] = 1µg/L, initial pH 5.1, agitation speed= 90 rev.min-1. Symbols: ● = studied medium, o = blank medium

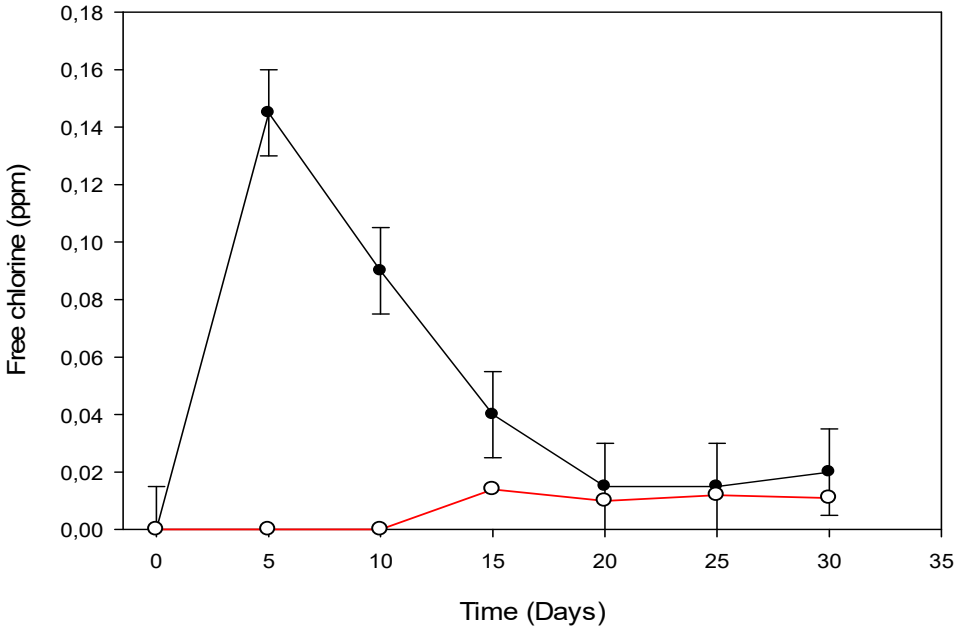

Fig. 5. Free chlorine released during Bacillus sp growth in liquid medium; T=27±2°C, [chlordecone] = 1µg/L, initial pH 5.1, agitation speed= 90 rev.min-1. Symbols: ● = studied medium, ○ = blank medium

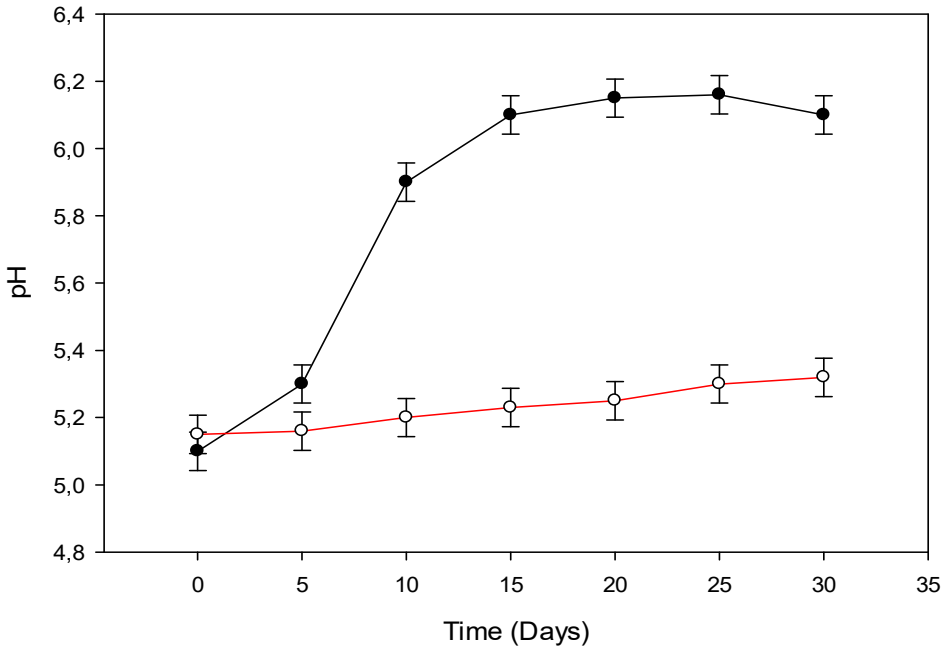

Fig. 6. pH variation during Bacillus sp growth in liquid medium; T=27±2°C,[chlordecone]= 1µg/L, initial pH 5.1, agitation speed= 90 rev.min-1. Symbols:● = studied medium, ○= blank medium

Figure 6 shows the evolution of pH during incubation of *Bacillus* sp with chlordecone. This graph presents 03 steps; especially, a pH increase during the first decline phase of the bacterial biomass, followed by a second exponential increase and finally, a stabilization of the pH as last phase.

4 DISCUSSION

Texture analysis of the soil sampled are similar to those found of the agronomic horizon (0 - 20 cms) of certain agricultural soils notably the andosols with about 59.7% and 79.7% of sand [7]. This type of soil is met in volcanic region with ashes as rock source. These grounds present high allophane content (an amorphous material constituted by silica, alumina and iron). The Southwest Cameroon region soils are considered as volcanic [3], [31], and as it is generally the case of Andosols, the studied microcosm is also acid. Indeed, this acidity also allows characterizing quantity of allophane. So, for pH between 4 and 5, allophane content of the sample is raised. Besides, these soils are rich in organic matter (OM ≥ 2 $mg.L^{-1}$) and thus, hold a very high adsorption capacity. This OM content would favour the retention of pesticides, OCP in particular. Indeed, it was shown that andosols have the biggest capacity of CLD retention than other classes of soils [32]. In addition to high CLD retention capacity, these soils present a low power of contamination of other environmental compartments by washing and streaming. Notwithstanding, this low power of contamination would lengthen the duration of pollutants transfer from contaminated soil towards aquatic circles and would cause a lasting pollution by the OCP in particular.

Concerning soil mineral analysis, obtained results are related to agricultural ground concerned by banana cultures. Indeed, potassium is particularly important for this type of fruits and is generally added during soil amendment. Sodium has a less importance because excess of sodium is harmful to banana tree [31], [33]. Chlorine has the same effect and is not necessarily added to soil although chlorine toxicity levels are very variable according to the variety of banana [31], [33]. Thus, the chlorine content observed could be related to persistent chlorinated compounds used on this soil during the previous decades. Because of their persistent character, these contrary compounds would be slowly dechlorinated and would maintain a continuous chlorine concentration in the ground. Consequently, microorganisms isolated from this soil would be already adapted to high chlorine content environment.

During *Bacillus* isolate incubation with CLD, the first decline step may be considered as a lag phase. Indeed, biodegradation of organic chemicals sometimes requires an acclimatization period of the microorganism before any use of the substrate [34], [35]. During this lag phase, little or no biodegradation is observed and microbial population produces enzymes necessary for conversion of an unusual substrate. Though microorganism in our case have been isolated from soil owning a CLD uses history, the *Bacillus* strain assayed requires an acclimatization phase, showing that this compound is not a preferential substrate for his metabolism and/or reveals the refractory character of this organochlorine for biodegradation by microorganism inoculated.

Concerning free chlorine analyses, an unexpected result has been chlorine released during the first decline phase. This dechlorination can be explain such as, during lag phase, a little biodegradation may take place and the dehalogenase enzymatic system produced could be act in the medium for CLD dechlorination [34]. Similar behaviour have been observed with *Fusarium verticillioides* in minimal salt medium [36]. In this case, lindane degradation has been monitored though the number of microorganism colonies decreased.

Due to the only one major peak of chlorine released, second and third bacterial biomass rises can be assigned to cryptic growth. The decrease of bacterial biomass would be understandable by microbial cells autolysis due to unfavourable culture medium. Indeed, during decline phase, bacteria division is strongly reduced or interrupted, many of them die and are destroyed by autolysins. Acclimatization period, nutriments unavailability or the accumulation of toxic metabolites due to not renewed medium would justify the death of cells. In our case, the unavailability would concern the cell incapacity to use the overall chlordecone present in the medium. Thus, microbial multiplication after 15 days could be assigned to a cryptic growth and would be justified by the presence of numerous organic compounds coming from the cytoplasmic contents of cells and used by the surviving microorganism as carbon source [37]. Moreover, after the cryptic period, death of cells could be due to the negative effect of pH increase, out of accumulation of toxic metabolites. Indeed, as observed with experimental design, pH owns a negative effect on the isolated *Bacillus* strain growth. It should be noticed that neutral pH is generally considered optimal for metabolic activities of the genus *Bacillus*, but it is not the case in our study.

5 CONCLUSION

This study suggested that microbial degradation of chlordecone did occur under aerobic conditions with Bacillus strain. This bacterium has been isolated from Cameroonian ecosystem owning organochlorine pesticides uses story. CLD biodegradation potential are influenced by pH of the medium and incubation temperature. Biodegradation pathway seems to be a double dechlorination only.

Further study on monitoring mineralization of chlordecone, identification of the intermediate products and the specific microbial community involved are required to determine the exact dechlorination pathway biodegradation of chlordecone in these aerobic conditions.

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REFERENCES

[1] S. Legeay, P.-A. Billat, N. Clere, F. Nesslany, S. Bristeau, S. Faure and C. Mouvet, 'Two dechlorinated chlordecone derivatives formed by in situ chemical reduction are devoid of genotoxicity and mutagenicity and have lower proangiogenic properties compared to the parent compound', *Env. Sci Pollut Res*, Published online, pp 11, 2017.

[2] A. Picot and M. Rabache, 'FICHE TOXICO ECOTOXICO CHIMIQUE N° 3: Chlordecone (Kepone)'. Association Toxicologie-CNAM, 2005.

[3] J. N. Okolle, G. H. Fansi, F. M. Lombi, P. S. Lang, and P. Loubana, 'Banana entomological Research in Cameroun: How Far and What Next?', *Afr. J. Plant Sci. Biotechnol.*, vol. 3, no. 1, pp. 1–19, 2009.

[4] PNUE, 'Chlordecone: Projet d'Evaluation de la Gestion des Risques'. http://www.pops.int/documents/meetings/poprc_3/meetingdocs/poprc3_doc/10/K0762894.F_POPS_POPRC_3_10.pd, 2007. Accessed September 2016.

[5] J.-Y. Le Deaut and C. Procaccia, 'Les impacts de l'utilisation de la chlordécone et des pesticides aux Antilles : bilan et perspectives d'évolution'. Assemblée Nationale Française: Office Parlementaire d'Évaluation des Choix Scientifiques et Technologiques, France, no 1778, pp 223, 2009.

[6] O. Boucher, M-N*. Simard, G. Muckle, F. Rouget, P. Kadhel, H. Bataille, V. Chajes, R. Dallaire, J-P. Thome, L. Multigner and S. Cordier*, 'Exposure to an organochlorine pesticide (chlordecone) and development of 18-month-old infants', *NeuroToxicology*, vol. 35, pp. 162–168, 2013.

[7] J. D. Fernandez-Bayo, C. Saison, M. Voltz, U. Disko, D. Hofmann, and A. E. Berns, 'Chlordecone fate and mineralisation in a tropical soil (andosol) microcosm under aerobic conditions', *Sci. Total Environ.*, vol. 463–464, pp. 395–403, 2013a.

[8] W. L. Dilling, 'Bishomocubane Chemistry. 14. Molecular Mechanics Calculations on Bishomocubyl Systems', *J Org Chem*, vol. 58, no. 20, pp. 5338–5344, 1993.

[9] PNUE, 'Convention de Stockholm sur les Polluants Organiques Persistants (POP)'. Secrétariat de la Convention de Stockholm, 2009.

[10] N. Cimetiere, S. Giraudet, M. Papazoglou, H. Fallou, A. Amrane, and P. LeCloirec, 'Analysis of chlordecone by LC/MS–MS in surface and wastewaters', *J. Environ. Chem. Eng.*, vol. 2, pp. 849–856, 2014.

[11] A. Durimel, S. Altenor, R. Miranda-Quintana, P. Couespel Du Mesnil, U. Jauregui-Haza, R. Gadiou and S. Gaspard., 'pH dependence of chlordecone adsorption on activated carbons and role of adsorbent physico-chemical properties', *Chem. Eng. J.*, vol. 229, pp. 239–249, 2013.

[12] S. Gaspard, L. Laquitaine, and A. Durimel, 'Recherche d'une voie de dépollution des milieux et matériaux contaminés par la chlordécone et l'hexachlorocychlohexane: Remédiation à la pollution par la chlordécone aux Antilles.', *Les Cahiers du PRAM*, vol. 9–10, pp. 21–23, 2011.

[13] Y.-M. Cabidoche, M. Jannoyer, and H. Vanniere, 'Pollution par les organochlorés aux Antilles, Conclusions du Groupe d'Etude et de Prospective', Centre de coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) et Institut National de la Recherche Agronomique (INRA) France, 66p, 2006.

[14] Y.-M. Cabidoche and M. Lesueur-Jannoyer, 'Pollution durable des sols par la chlordécone aux Antilles : comment la gérer ?', *Innov. Agron.*, vol. 16, pp. 117–133, 2011.

[15] V. Rana, R. Kissner, U. Jauregui-Haza, S. Gaspard, and J. Levalois-Grützmacher, 'Enhanced chlordecone (Kepone) removal by FeO-nanoparticles loaded on activated carbon', *J. Environ. Chem. Eng.*, vol. 5, no. 2, pp. 1608–1617, 2017.

[16] J. J. Gamboa-Carballo *et al.*, 'Theoretical study of chlordecone and surface groups interaction in an activated carbon model under acidic and neutral conditions', *J. Mol. Graph. Model.*, vol. 65, pp. 83–93, 2016.

[17] V. Rana, R. Kissner, S. Gaspard, and J. Levalois-Grützmacher, 'Cyclodextrin as a Complexation Agent in the Removal of Chlordecone from Water', *Chem. Eng. J.*, vol. 293, pp. 82–89, 2016.

[18] P. LeCloirec, 'Traitements des eaux; Elimination de la Chlordécone par adsorption sur charbon actif, Etats des lieux et prospectives: Remédiation à la pollution par la Chlordécone aux Antilles', *Les Cahiers du PRAM*, vol. 9–10, pp. 17–19, 2011.

[19] S. Malato, 'Dégradation de la Chlordécone dans l'eau en utilisant des Procédés d'Oxydation Avancée (AOPS): Remédiation à la pollution par la chlordécone aux Antilles.', *Cah. PRAM*, vol. 9–10, pp. 13–16, 2011.

[20] H. Macarie and J. Dolfing, 'La chlordécone (CLD) est-elle véritablement réfractaire à une dégradation microbienne ? Remédiation à la pollution par la chlordécone aux Antilles', *Les Cahiers du PRAM*, vol. 9–10, pp. 25–30, 2011.
[21] M. Devers, E. Topp, N. Rouard, and F. Martin-Laurent, 'La biodégradation de la chlordécone est-elle impossible? Remédiation à la pollution par la chordécone aux Antilles', *Les Cahiers du PRAM*, vol. 9–10, pp. 41–44, 2011.
[22] S. Orndorff and R. R. Colwell, 'Microbial Transformation of Kepone', *Appl. Environ. Microbiol.*, vol. 39, no. 2, pp. 398–406, 1980.
[23] S. Pesce, 'Biodegradation et bioremédiation de la chlordécone - Approches de recherches possibles: Remédiation à la pollution par la Chlordécone aux Antilles', *Les Cahiers du PRAM*, vol. 9–10, pp. 45–47, 2011.
[24] W. Achouak, 'Devenir de la chlordécone dans l'environnement: Remédiation à la pollution par la Chlordécone aux Antilles: Remédiation à la pollution par la chlordécone aux Antilles', *Les Cahiers du PRAM*, vol. 9–10, pp. 49–52, 2011.
[25] I. S. de S. P. ISSeP, 'S-II-12V2 – Extraction des éléments disponibles dans les terres agricoles'. CWEA, 2014.
[26] H. M. Murthy and H. K. Manonmani, 'Aerobic degradation of technical hexachlorocyclohexane by a defined microbial consortium', *J. Hazard. Mater.*, vol. 149, pp. 18–25, 2007.
[27] H. Kiyohara, K. Nagao, and K. Yana, 'Rapid screen for bacteria degrading Water-Insoluble, solids Hydrocarbons on Agar plates', vol. 43, no. 2, pp. 454–457, 1982.
[28] S. F. Pesce and D. Wunderlin, 'Biodegradation of Lindane by a native bacterial consortium isolated from contaminated river sediment', *Int. Biodeterior. Biodegrad.*, vol. 54, pp. 255–260, 2004.
[29] G. Zheng, J. Selvam, and W. C. Wong, 'Rapid degradation of lindane (γ-hexachlorocyclohexane) at low temperature by Sphingobium strains', *Int. Biodeterior. Biodegrad.*, vol. 65, pp. 612–618, 2011.
[30] A. Warth, 'Relationship Between the Heat Resistance of Spores and the Optimum and maximum Growth Temperatures of Bacillus Species', *J. Bacteriol.*, vol. 134, no. 3, pp. 699–705, 1978.
[31] A. Lassoudière, *Le bananier: Un siècle d'innovations techniques*. Editions Quae, 2012.
[32] Y.-M. Cabidoche, R. Achard, P. Cattan, C. Clermont-Dauphin, F. Massat, and J. Sansoulet, 'Long-term pollution by chlordecone of tropical volcanic soils in the French West Indies: A simple leaching model accounts for current residue', *Environ. Pollut.*, vol. 157, pp. 1697–1705, 2009.
[33] M. Belfakih, M. Ibriz, A. Zouahri, and S. Hilali, 'Effet de la salinité sur la croissance des deux variétés de bananier « grande naine » et « petite naine » et leur nutrition minérale au Maroc', *J. Appl. Biosci.*, vol. 63, pp. 4689 – 4702, 2013.
[34] S. Cuozzo, M. Fuentes, N. Bourguignon, C. Benimeli, and M. Amoroso, 'Chlordane biodegradation under aerobic conditions by indigenous Streptomyces strains', *Int. Biodeterior. Biodegrad.*, vol. 66, pp. 19–24, 2012.
[35] T. Hirano, T. Ishida, K. Oh, and R. Sudo, 'Biodegradation of chlordane and hexachlorobenzenes in river sediment', *Chemosphere*, vol. 67, pp. 428–434, 2007.
[36] F. Guillen-Jimenez, E. Cristiani-Urbina, J. C. Cancino-Diaz, J. L. Flores-Moreno, and B. Barragan-Huerta, 'Lindane biodegradation by the Fusarium verticillioodes AT-100 strain, isolated from Agave tequilana leaves: Kinetics and identification of metabolites', *Int. Biodeterior. Biodegrad.*, vol. 74, pp. 36–47, 2012.
[37] S. Hogg, *Essential Microbiology*. John Wiley § Sons, Ltd, 2005.