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Anaerobic Biodegradation of Sumithion an Organophosphorus Insecticide Used in Burkina Faso Agriculture by Acclimatized Indigenous Bacteria

^{1,2}Paul W. Savadogo, ²Aly Savadogo, ²Aboubacar S. Ouattara, ¹Michel P. Sedogo and ²Alfred S. Traoré

¹Laboratoire Sol Eau Plante, Institut de l'Environnement et de Recherches Agricoles
01 BP 476 Ouagadougou 01, Burkina Faso

²Laboratoire de Biochimie/Microbiologie, Centre de Recherche en Sciences Biologiques, Alimentaires et
Nutritionnelles, UFR-SVT, Université de Ouagadougou, 03 BP 7131 Ouagadougou 03, Burkina Faso

Abstract: Biodegradation of Sumithion (o,o-dimethyl o-(3-methyl-4-nitrophenyl) phosphorothioate) was tested using microorganisms isolated from mud under controlled conditions. Results showed that after 2 years of acclimatation, the indigenous microorganisms can biodegrade sumithion under anaerobic conditions. This biodegradation lead to methane production. Biodegradation kinetics monitored by high performance liquid chromatography and gas chromatography showed that the acclimated consortium is able to carry out the biodegradation of 20, 50 and 100 mg L⁻¹ of Sumithion, respectively in 15, 25 and 45 days. The end products of sumithion biodegradation were gases. Such as CO₂, N₂, CH₄ and H₂S. Isolation and partial characterization of a strain able to degrade Sumithion were realized. This strain named SY can be easily produced and used for the bioremediation of waters and soils contaminated by Sumithion.

Key words: Biodegradation, sumithion, acclimatation, pesticides, Burkina Faso

INTRODUCTION

The extensive use of pesticides in agriculture is compromising soil and water quality. Burkina Faso agriculturists like others ones in the developing countries uses more chemical substances in order to improve their agricultural output (Cissé *et al.*, 2001). The use of chemical products permits to fertilize the fields and to fight against the devastating of crops. But when these products remain in the fields they can provoke some damages to the environment. It is particularly the case of pesticides residues which fall on the soil during the treatments and remain more or less a long time according to their remanence (Aslan and Turkman, 2006; Cerrillo *et al.*, 2005; Friberg-Jensen *et al.*, 2003; Pazou *et al.*, 2006; Shukla *et al.*, 2006).

A possible way to solve the problems of pesticide waste, washings or contamination of soil by spillage is bioremediation with pesticide-degrading microorganisms. Biodegradation is a fundamental attenuation process for pesticides in soil (Guo *et al.*, 2000; Barahona *et al.*, 2004; Bardi *et al.*, 2003). This process catalyzed by soil microbes is governed by both abiotic and biotic factors. In spite of their toxicity, several microorganisms are capable of mineralizing or transforming nitroaromatics. p-Nitrophenol (PNP) is widely used in the manufacture of pesticides,

pharmaceuticals and dyes. It is also the major metabolite resulting from the microbial degradation of parathion and methyl parathion, organophosphate pesticides widely used as agricultural insecticides in several third world countries. Microbial degradation of PNP has been reported for several bacteria including *Flavobacterium*, *Pseudomonas*, *Moraxella*, *Arthrobacter* and *Bacillus* (Spain and Van Veld, 1983; Radosevich *et al.*, 1997). Among the methods used to solve the problem of environment contamination by chemical substances we recommend the use of effective microorganisms capable to eliminate the residues of pesticides in soil. Indeed after an adequate acclimatation some microorganisms become capable to use the pesticides as a natural substrate. Such microorganisms introduced in contaminated soil are going to clean it of pesticides residues. Several reviewed showed that bioremediation, which involves degradation of target chemical by microbial cells, is used to clean up sites contaminated by pollutants such as pentachlorophenol, parathion, 2,4,5-T, atrazine, herbicides, polyaromatic hydrocarbons (Barles *et al.*, 1979; Pavlostathis *et al.*, 2003; Barbeau *et al.*, 1997; Mathava and Ligy, 2006; Verma *et al.*, 2006).

Sumithion is among the pesticides the more used in agriculture in Burkina Faso and involved in the contamination of cotton zones (Savadogo *et al.*, 1999; Savadogo, 2001).

However cotton constitutes the first agricultural resource of Burkina Faso and is very important for financial independency of the peasants. In this condition it is urgent to think about some means in order to minimize the bad effects of pesticides used.

The present study shows the results gotten on anaerobic biodegradation of Sumithion with an inoculum of microorganism acclimatized on sumithion and able to degrade this pesticide in soil.

MATERIALS AND METHODS

Media, Culture condition and microorganisms isolation

strains isolation: The inoculum for Sumithion biodegradation study has been gotten from samples of mud in the purification station of Ouagadougou slaughterhouse. This station was an anaerobic lagoon functioning in methanogenic condition. For isolation the anaerobic culture media has been prepared according to the technics described by Hungate (1969) and modified by Miller and Wolin (1974). The culture media are degassed while carrying them to boiling, cooled under flow of nitrogen free from oxygen, distributed in the serum bottles, Hungate tubes or high-pressure tubes, then sterilized by autoclaving at 110°C during 45 min. Roll-tube technic developed by Hungate (1969) has been used for isolation of bacterial strains on anaerobic solid media. Prior to inoculation, media are heated to make melt the agar and maintained at 48°C. Inoculation has been made with 0.5 mL of culture media per tube. The following culture media were prepared and used.

Basal culture medium: It contains neither source of carbon nor electron acceptor and is consisted of three sterile anaerobic solutions (A, B, C). The mineral solution (A) contains: K_2HPO_4 , 0.35 g; KH_2PO_4 , 0.25 g, NaCl, 0.5 g; NH_4Cl , 2 g; Yeast extract, 0.1 g; Trace elements of Balch *et al.*, (1979), 1 mL; Resazurin (0.1%), 1 mL; air free distilled water, qsp 1000 mL. The pH was adjusted to 7.2 ± 0.2 . The reductor solution (B) contains 2.5 g L^{-1} of $Na_2S \cdot 9H_2O$ in air free distilled water. The buffer solution (C) contains 4 g L^{-1} of $NaHCO_3$ in air free distilled water. The complete culture medium is achieved by adding to A, 2% (V/V) of B and 2% (V/V) of C prior to inoculation.

Balch *et al.* (1979) trace element solution: The composition of this solution is as follow: nitroacetic acid, 1.5 g; $MgSO_4 \cdot 7H_2O$, 3.0 g; NaCl, 1.0 g; $MnSO_4 \cdot 2H_2O$, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.1 g; $CoCl_2 \cdot 6H_2O$, 0.1 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; $ZnCl_2$, 0.1 g; $CuSO_4 \cdot 5H_2O$, 0.01 g; $AlK(SO_4)_2$, 0.01 g; H_3BO_3 , 0.01 g; $Na_2MoO_4 \cdot 2H_2O$, 0.01 g; H_2O , qsp 1000 mL.

Widdel *et al.* (1983) trace element solution: The composition of this solution is as follow: $FeCl_2 \cdot 4H_2O$, 1.5 g, HCl (25%), 10.0 mL; $CoCl_2 \cdot 6H_2O$, 0.19 g; $MnCl_2 \cdot 4H_2O$, 0.1 g; $ZnCl_2$, 0.07 g; H_3BO_3 , 0.006 g; $Na_2MoO_4 \cdot 2H_2O$, 0.036 g; $NiCl_2 \cdot 6H_2O$, 24 mg; $CuCl_2 \cdot 2H_2O$, 2 mg; distilled H_2O , qsp 1000 mL. Both Widdel *et al.* (1983) and Balch *et al.* (1979) trace elements solution are kept to 4°C.

Bacteria isolation and incubation: The pesticide, an electrons acceptor and a reducer of potential were added in anaerobic and sterile conditions in Hungate tubes containing basis medium for bacteria. The addition of a complementary source of energy is achieved in the same way. Then, the tubes are inoculated and incubated at 37°C.

Morphological and physiological studies: The morphological study has been achieved with an optic microscope (CARL ZEISS, West Germany). The range of temperature permitting the growth of the strains was determined while measuring the maximal speed of growth (μ_{max}) at different temperatures in thermostated water bath. For this study, the cultures were achieved in Hungate tubes containing the basal medium with 20 mg L^{-1} of pyruvate or glucose. The growth was followed while measuring directly the optical density of the growth tubes (DO) at 580 nm with a spectrophotometer (Miltonroy, Spectronic 601). Cultures were re-inoculated monthly with 10% (V/V). During the exponential growth phase, the tube was used to inoculate three new tubes from which the kinetics of the growth was followed. The average of the three maximal growth rate (μ_{max}) for each temperature was calculated. The range of pH permitting the growth of the strains was determined while measuring the maximal growth rate of the strain at different pH as described previously.

Estimation of bacterial growth: The dosage of bacterial proteins was carried out according to the method of Lowry *et al.* (1951). Protein concentration was determined using a calibration curve established with the bovine serum albumin ($Y = 0.018X + 0.029$; $R = 0.996$).

Estimation of inocula efficiency to sumithion degradation: Inocula efficiency to sumithion degradation under anaerobic conditions was estimated by methane production capacity (Savadogo, 2001; Shelton and Tiedje, 1984a,b). Inocula gotten from six sites were tested. It was about inocula gotten from EIER waste water (EIER), the effluents of tannery (Tannerie), Ouagadougou slaughterhouse waste water

(slaughterhouse), Ouagadougou dam (dam) and two waste water from Zogona (Zogona 1 and Zogona 2). Inoculation was done with the presence of 20 mg L⁻¹ of sumithion under methanogenic conditions and incubated at 37°C in the dark.

Biodegradation measurements

Monitoring of sumithion biodegradation in soil

Soil treatment: Appropriated soil was gotten from Ouagadougou university experimental field. Free air dried soil sieved through a 2 mm sieve was introduced into a 5 L plastic pot and floods with 1.5 L of sterile distilled water in order to have a water column of about 25 mm. To estimate the chemical degradation soil was sterilized by autoclaving 3 times at 121°C during 1 h.

Sumithion extraction and analysis: Sumithion extraction was performed by shaking three times 10 g of soil in 40 mL of diethyl ether-chloroform (1:1) during 45 min. After a centrifugation at 10000 g during 15 min and filtration with Millipore membrane (0.22 µm), sumithion was analyzed by HPLC. The samples were kept at -20°C in the dark until analysis. Chromatograph with an apolar column C₁₈ Symmetry Waters, coupled to an UV detector (Jasco, UV-975, Japan) and to an integrator (Hewlett-Packard) was used for pesticide quantification. The analysis conditions were as follow:

Eluant: Six hundred milliliter of acetonitrile + 400 mL of distilled water filtered on millipore membrane (0.22 µm); volume of injection, 20 µL; flow of the pump (Jasco, PU-980, Japan): 0.9 mL min⁻¹; column temperature: ambient temperature (23±2°C); detection at 240 nm.

The samples were diluted in the eluant to get a compatible concentration with the range of standards used prior to injection.

Analysis of sumithion aromatic degradation products:

HPLC chromatograph (Hewlett Packard) model 1100 with C₁₈ SYMMETRY column (Waters Chromatography) coupled to a computer (Hewlett-Packard) equipped with the Chemstation software was used. The analysis conditions were as follow: 1 mL of culture medium was put in an Ependorf tube. Then 100 µL of pure acetic acid was added. After centrifugation at 13000 g during 15 min (Biofuge 13, Heræus) the solution was filtered through a millipore filter (0.2 µm). The inject volume was 25 µL. The detection wavelength was fixed to 240 nm. The flow was 0.75 mL min⁻¹. Column temperature was fixed to 35°C. Mobile phase was constituted of a gradual mixture of two solvents A and B. The parameters of this mixture were constructed in a computer program, especially conceived

for the analysis of the aromatic compounds. The composition for 1 L of solvent A was 800 H₂O mL filtered on Millipore membrane (0.45 µm), 200 mL of pure acetonitrile and 500 µL of pure acetic acid. The B solvent was pure acetonitrile for HPLC.

Analysis of volatile fatty acids and sulfides production:

HPLC chromatograph (Thermo Separation Products; California, USA) with an ORH 801 column (Interaction Chemicals, USA) was used. The detector was a refractometer RID 6A (Shimadzu Co., Kyoto, Japan). The injection volume was 20 µL. The chromatograph was coupled to a Chromatopac C-R6A integrator (Shimadzu CO., Kyoto, Japan). The analysis conditions were as follow. The mobile phase was H₂SO₄ solution 0.005 N filtered (0.22 µm, Millipore) then air free. The eluant flow was 0.6 mL min⁻¹. The column temperature was 35°C. Prior to injection, the sample was centrifuged at 13000 g during 15 min then diluted after filtration by using the suitable solvent. Sulfides production was measured by the method described by Cord-Ruwisch (1985).

Analysis of the gaseous products (CH₄, CO₂, N₂):

The gaseous products have been analyzed by gaz chromatography with catharometric detection using a Girdel chromatograph serial 30, Type 30-C-TP N°900 equipped with a recording Servotrace Type PL N°9349 and Altech CAT N°8700 column. Gases analysis was done by manual injection of 0.5 mL of gaseous phase. The areas of the detected peaks permit to calculate the concentration of every gas in the mixture by comparison with the concentrations calculated from the peaks of corresponding standards. The analytical conditions were as follows: vector gas (argon at 1 bar), injection and detection temperatures (100°C), column temperature (60°C).

RESULTS AND DISCUSSION

Inoculum acclimatation: After 60 days of incubation, the production of methane was noted with inocula gotten from Ouagadougou slaughterhouse, waste water from zogona and waste water from tannery. Methane production was not recorded in the sterilized medium containing 20 mg L⁻¹ of sumithion (Table 1). Inoculum from Ouagadougou slaughterhouse gave better activity in methane production (35 µmole mL⁻¹). The amount of methane produced was weak during the first ten days then it increased quickly between the 10 and the 35 days and finally stopped after 50 days of incubation (Table 1).

Pesticides toxicity and microbial activity are two important parameters for methane production

Table 1: Production of methane with different inocula

Days after incubation	Eier	*Concentration of methane in the gaseous phase ($\mu\text{mole mL}^{-1}$)				
		Ouagadougou dam	Zogona 1	Zogona 2	Tannery	Slaughterhouse
0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.6	0.0	0.0	10.8
10	0.0	0.0	2.4	0.0	0.0	13.6
15	0.0	0.0	4.6	0.4	0.2	28.8
20	0.0	0.0	4.4	0.4	0.4	31.2
25	0.0	0.0	4.6	0.4	0.6	31.6
30	0.0	0.0	4.6	0.6	0.6	32.4
35	0.0	0.0	4.6	0.8	0.8	36.2
40	0.0	0.0	4.6	0.8	0.8	36.0
45	0.0	0.0	4.6	0.8	0.8	35.6
50	0.0	0.0	4.6	0.8	0.8	35.0
55	0.0	0.0	4.6	0.8	0.8	35.0
60	0.0	0.0	4.6	0.8	0.8	35.0

*The experiences have been carried out in serum bottles of 50 mL containing 27 mL of culture medium inoculated with 3 mL of inoculum and have been incubated to 37°C in the dark. Each value of the table is an average of 6 measurements

Table 2: pH and total Volatiles Fatty Acids (VFA) evolution in the medium during the first month of acclimatation

Days after incubation	pH of the medium containing 20 mg L ⁻¹ of Sumithion	Volatile Fatty Acids (mg L ⁻¹)
0	7.26	280±10
5	7.10	360±16
10	7.05	300±12
15	6.60	280±90
20	6.70	240±80
25	7.10	260±15
30	7.20	240±15

The experiences have been carried out in serum bottles of 500 mL containing 390 mL of culture medium inoculated with 40 mL of inoculum and have been incubated to 37°C in the dark. The indicated values are averages from three measures

(Donlon *et al.*, 1995). A better capacity to pesticides degradation results in a strong production of methane since methanogene bacteria are at the end of trophic chain in the microorganisms consortia (Bechard *et al.*, 1996). Microbial diversity plays also a significant role in the biodegradation of xenobiotic molecules such as synthetic pesticides (Jim and Van Veld, 1983). The inoculum gotten from Ouagadougou slaughterhouse was retained for the study aiming at the optimization of sumithion biodegradation.

Optimization of sumithion biodegradation with two years acclimated consortium: During the two years acclimatation period, the incubation temperature and the initial pH were respectively 7.2 and 37°C. The evolution of the pH was followed during the whole incubation period. Results showed that the pH lowers slightly between the 1st and 5th day of incubation with Sumithion (Table 2). During the first month there was an important decrease of pH between the 10th and the 20th day of acclimatation (Table 2). That could correspond to an accumulation of volatile fatty acids from degradation of Sumithion. (Table 2) After the 20th day the pH increased and reached 7.2 at the 30th day of incubation. During the acclimatation the pH was located between 6.5 and 7.7 and favorable to

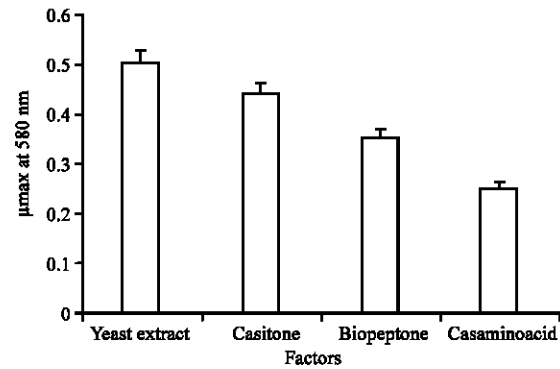


Fig. 1: Effect of various factors on the growth of bacteria of 2 years acclimated inoculum with 20 mg L⁻¹ of sumithion

a good methanogenesis activity. Consequently no particular measure has been taken to adjust the pH. The measurement of total Volatile Fatty Acids (VFA) carried out during the first month of the acclimatation showed an increase of the initial concentration from 280 to 360 mg L⁻¹ during the first 5th days of incubation (Table 2). However the rate of VFA drops after 10 days. According to Lagrange (1980) and Traoré (1992), the concentration of VFA in the culture media remained in the favourable range to a good microbial activity (200 and 700 mg L⁻¹).

To achieve the biodegradation of xenobiotics some bacteria require the presence of growth factor. Thus four growth factors have been tested for their suitability to microbial growth. It was about yeast extract (Sigma-type), casitone (Difco), biopeptone (Difco) and casaminoacides (Difco). Results showed that yeast extract is the best growth factor for bacteria involved in the biodegradation of sumithion (Fig. 1).

Our results showed that during the acclimatation, the rate of sulphides was between 40 and 60 mg L⁻¹ (Table 3).

Table 3: Production of sulphides with the two years acclimated inoculum

Days after incubation	Sulphides concentration in the medium (mg L ⁻¹)
0	40±4
5	47±4
10	50±3
15	55±2
20	60±4
25	58±3
30	50±2

Average of 3 measures ± ecartype

In metanogenic conditions (without addition of exogenous electrons acceptor) the production of sulphides is very weak and doesn't reach 100 mg L⁻¹ which is the value not to exceed for a good methanogenesis (Soren and Ahring, 1992).

Our previous study had shown that the growth of the bacteria involved in the biodegradation of Sumithion is better with yeast extract and casitone (Savadojo, 2001). These results are similar to those of Moore *et al.* (1983) which showed that thiamine was the essential growth factor of yeast extract for two bacterial strains involved in Glyphosate biodegradation. Indeed yeast extract is rich in several vitamins. During our study, yeast extract and casidone concentration was 0.1 mg mL⁻¹ of culture medium. Such concentration is low and could not support microorganism's growth and so sumithion was the sole carbon source in the medium.

Partial identification of a bacterial strain able to degrade sumithion: During the acclimation, the bacteria able to resist and growth in presence of sumithion proliferate while the other forms disappear. In our case, the cocci and the long rods seem to be active in the biodegradation of Sumithion. After two years of acclimation it was noted that acclimated inoculum offered a rapid biodegradation of Sumithion. A strain named SY has been isolated from the sumithion degrading consortium. Some morphological and physiological characteristics of SY is given in Table 4. Strain SY is a facultative anaerobic rod with a negative Gram staining. SY is a spore forming bacteria, positive catalase. It was tentatively identified as the genus *Bacillus* (Bergey's Manual of Systematic Bacteriology, 2001). SY can be easily cultivated on a medium supplemented with glucose at 37°C and pH 7 in order to obtain a great quantity of inoculum. This inoculum could be used for waste water and contaminated soils treatment (Table 4).

Kinetic of Anaerobic biodegradation of sumithion with the two years acclimated consortium: The degradation of Sumithion at concentration of 20 mg L⁻¹ is very fast and achieved in 15 days with a speed of 1.3 mg L⁻¹ J⁻¹. At the end of 5 days of incubation 32% of the initial concentration of Sumithion is degraded and 76% at 10th, then 100% on the 15th day. No abiotic degradation of

Sumithion was noted after 15 days of incubation (Fig. 2a). With an initial concentration carried to 50 mg L⁻¹ the biodegradation of the Sumithion is achieved in 25 days with a speed of 2 mg L⁻¹ J⁻¹. Thus, 69% of the initial

Table 4: Morphological and physiological characteristics of strain SY

Parameters	Characteristics of SY
Form	Rod
Mobility	Slow to rapid serpentiform
Gram staining	Negative
Spores	Terminale spore
Catalase	Positive
Oxydase	Negative
Substrats utilised	Glucose, fructose, arabinose, maltose, Mannitol, soluble starch, Pyruvate, acetate
Electrons acceptor (facultatif)	NaNO ₃
Range of temperature	15 to 45°C
Optimal temperature	37°C
Range of pH	5 to 9
Optimal pH	7

Media MB was used and the cultivation was in anaerobic condition using Hungate tubes. 20 mM of glucose was use as growth substrate for physiological studies

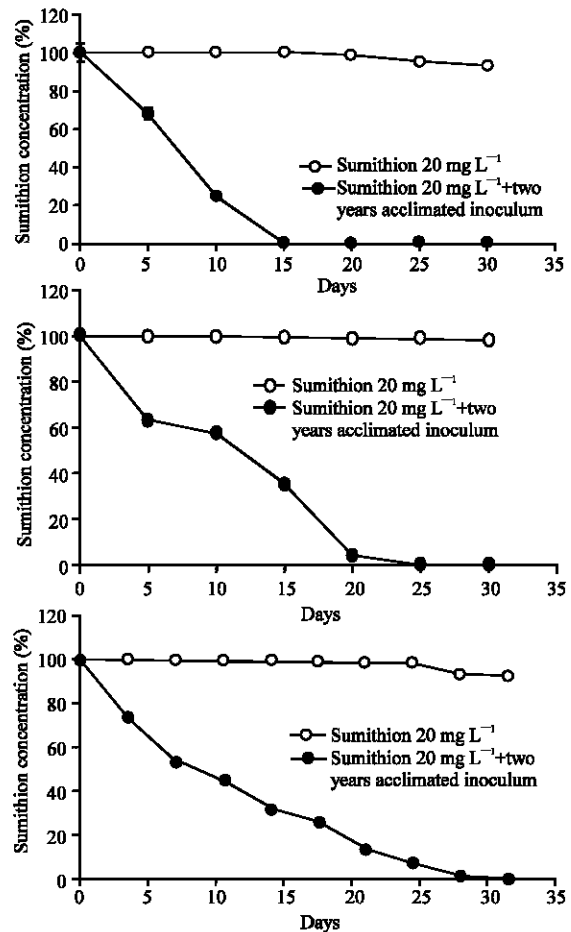


Fig. 2: Biodegradation of 20 mg L⁻¹ (A), 50 mg L⁻¹ (B) and 100 mg L⁻¹ (C) of sumithion by the two years acclimated inoculum

concentration of Sumithion was degraded in 5 days, 72% in 10 days. Between the 10th and the 20th day the degradation of the Sumithion is fast with a maximum speed of $2.7 \text{ mg L}^{-1} \text{ J}^{-1}$. Indeed, the initial concentration of Sumithion goes from 28.5 to 2 mg L^{-1} . (Fig. 2b) With initial concentration up to 100 mg L^{-1} , the speed of sumithion degradation was slow compared to those of 20 and 50 mg L^{-1} . Forty five days was necessary for the degradation of total amount of Sumithion. So that the speed of degradation was $2.2 \text{ mg L}^{-1} \text{ J}^{-1}$. The abiotic biodegradation was 4.7%. (Fig. 2c). These results showed that the two years acclimated consortium was efficient for the three concentrations: 20, 50 and 100 mg L^{-1} .

Kinetic of anaerobic biodegradation of sumithion with the isolated and partially identified strains SY

Degradation of sumithion by the strain SY without supplemented substrate: The degradation of Sumithion by SY was fast and no lag time was observed (Fig. 3). Between the 1st and the 10th day of incubation the concentration of Sumithion went from 20 - 03 mg L^{-1} with a maximal speed of degradation of $1.7 \text{ mg L}^{-1} \text{ J}^{-1}$. After 20 days of incubation Sumithion degradation was total (Fig. 4). The growth of the strain SY begins without lag phase. This growth is fast between the 1st and the 10th day of incubation. The growth is slow between the 10th and the 25th day where it ends.

Degradation of the sumithion by the strain SY in presence of 20 mg L^{-1} of glucose: The growth of the strain SY is fast in presence of glucose (Fig. 4). This growth begins practically without lag phase. The growth of the strain SY is accompanied by a degradation of the sumithion.

The totality of the glucose is metabolized after 10 days of incubation. At the end of 10 days of incubation one notes a degradation of the sumithion of 71.5%. The maximal speed of degradation was $1.4 \text{ mg L}^{-1} \text{ J}^{-1}$. This degradation goes with a decrease of pH. Indeed, the pH goes from 7.2 - 5.8 the 10th day and to 3.8 the 20th day. The drop of the pH could be responsible of sumithion degradation after 15th days. Indeed Coulibaly and Smith (1990) clearly highlighted the role of acids solutions in the degradation of the pesticides.

The growth of strain SY stopped after 10 days of incubation. The degradation of Sumithion was inhibited until the 20th day and it started again and continued until the thirty-fifth day. In presence of glucose the average speed of degradation of the 20 mg L^{-1} of Sumithion by the strain SY is weak ($0.6 \text{ mg L}^{-1} \text{ J}^{-1}$) compared to absence of glucose ($1 \text{ mg L}^{-1} \text{ J}^{-1}$).

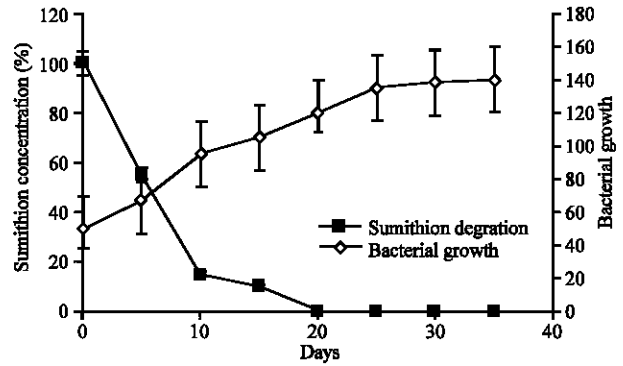


Fig. 3: Sumithion degradation and strain SY growth with sumithion (20 mg L^{-1}) as sole carbon source

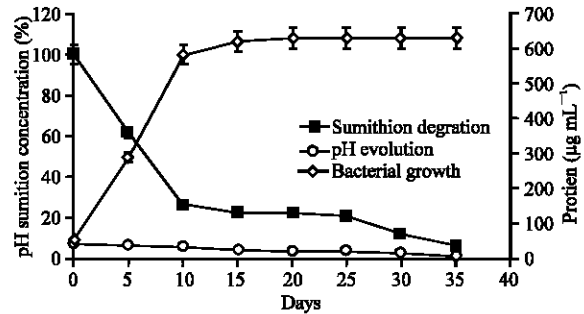


Fig. 4: Sumithion degradation and strain SY growth with sumithion (20 mg L^{-1}) in presence of 20 mg L^{-1} of glucose

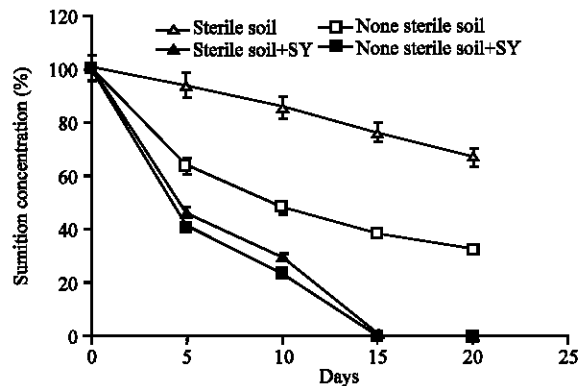


Fig. 5: Biodegradation of 20 mg L^{-1} of sumithion in inoculated and non inoculated soil

Biodegradation of Sumithion in soil by an inoculum of strain SY: Results of sumithion biodegradation in soil showed a better degradation of this pesticide with the strain SY. One recorded 100% of sumithion degradation at 20 days with SY against 33% without. These results showed that the sumithion degradation was due to biodegradation by SY (67%) (Fig. 5).

Before the inoculation of strain SY in soil it contained an indigenous microflora capable to breakdown pesticides. Indeed, without SY none sterile soil biodegraded 67% of sumithion in 20 days.

A similar study achieved by Alonso *et al.* (1997) showed that two bacterial strains (*Flavobacterium* sp. strain ATCC27551 and *Arthrobacter aureescens* strain

TW17) were involved in fenitrothion (active substance of Sumithion) and its hydrolysis product (3-methyl-4-nitrophenol) degradation in laboratory condition.

Sterile soil had also biodegradation activity. That is shown by sumithion degradation of 33%. Maybe the sterilization by autoclaving is not total. It can be the abiotic degradation due to volatilization or catabolism by

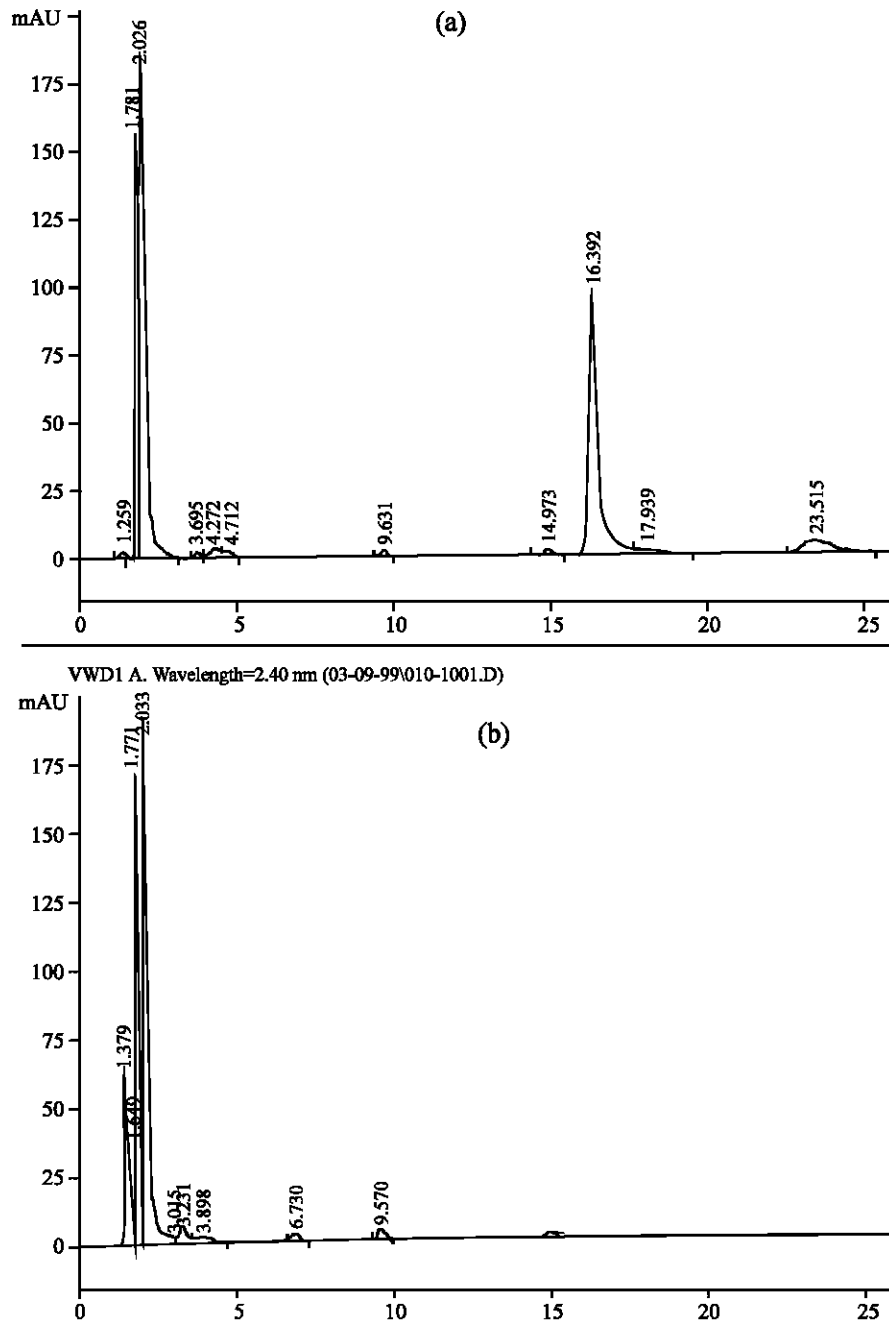


Fig. 6: Chromatogram of aromatic of sumithion biodegradation products after 10 days (a) and after 30 days (b) of incubation in presence of two years acclimated consortium

some substances of soil. Bastide *et al.* (1985) showed that the degradation of malathion was catalyzed on a complex argilo-humic-Cu²⁺ showing the particular role of the copper to pesticide biodegradation in soil (Bastide *et al.*, 1985).

Similar studies has been achieved on isolated microorganisms of soil and adapted to laboratory conditions in presence of pesticides. Results showed biodegradation of Isoproturon (86% in 72 h), Clortoluron (93% in 72 h) and fenitrothion (66% in 72 h). These microorganisms can be freeze-dried and can be used in the bioremediation of soils contaminated by these pesticides (Cernakova, 1995; Harison *et al.*, 1998; Mulbry *et al.*, 1996). What shows that the strain SY can be used in the resolution of the problems of contamination of soils by the sumithion.

A study of fenitrothion biodegradation achieved in an India soil showed that the biodegradation of this pesticide lead to metabolites coming from reactions as oxydative desulphuration, cleavage by hydrolysis of the P-O-Aryl link and a demethylation of the pesticide (Roy *et al.*, 1996). The study of the biodegradation of a pesticide in soil is indispensable if this last is destined to be used in agriculture. Indeed the knowledge of the half-life of the pesticide in soil is fundamental for modelling. The laboratory test offers a certain advantage insofar as it gives the possibility to establish the diagram of degradation and a precise balance of the elimination ways including the volatile fractions.

Metabolites of sumithion biodegradation: The sumithion biodegradation metabolites monitoring showed that this pesticide was completely degraded. Aromatic compounds analysis by HPLC showed after 10 days of incubation a degradation of Sumithion by bacterial consortium leading to the apparition of 8 peaks with a major peak at 16.39. But these peaks disappeared completely after 30 days of incubation what indicated that these molecules are intermediates (Fig. 6). A similar study achieved by Alonso *et al.* (1997) showed that a mediator of the anaerobic biodegradation of the fenitrothion by a strain *Flavobacterium* sp. ATCC 27551 was the 3-méthyl-4-nitrophénol. Gas Chromatography analysis showed that the end products of Sumithion degradation are gases. The CO₂, the CH₄ and the N₂ have been detected. It is evident from these results that use of inoculum with strain SY for anaerobic bioremediation of soils contaminated by sumithion could offer advantages insofar there is no accumulation of degradation products susceptible to contaminate the environment.

CONCLUSIONS

The results of our experiments clearly show that the inoculum acclimatized on the Sumithion can be used for the biodepollution of soil and waters contaminated by this pesticide. We report evidence for the existence of microorganisms like SY freshly isolated from natural ecosystems able to degrade pesticide. Although several hundred pesticides with different physical and chemical properties are widely used for agricultural purposes, our study was limited to one pesticide commonly used in Burkina Faso. More detailed monitoring studies have to be carried out on few others representative pesticides used in Burkina Faso.

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