METHOD OF DETOXIFICATION OF YPERITE BY USING HALOALKANE DEHALOGENASES

Application number: CZ 2005 – 352 A1
Date of submission: June 3, 2005

Abstract: The method of detoxification of yperite by the use of haloalkane dehalogenases or their compositions, the method of preparation of dehalogenating enzymes and of decontamination compositions which contain at least one wild type and/or modified haloalkane dehalogenase (EC 3.8.1.5) as an chemically active component. The preferred halogenases are DhaA from *Rhodococcus rhodochrous* NCIMB 13064, DmbA from *Mycobacterium bovis* 5033/66 or LinB from *Sphingomonas paucimobilis* UT26. Decontamination is utilized for detoxification of yperite from the surfaces of instrumentality, constructional objects, the people’s or animal’s skin and elements of environment, when yperite is exposed to the action of decontamination composition at +10 °C to +70 °C, preferably at +40 °C and pH = 4 to 12.

Applicant: Masaryk University, Žerotínovo nám. 9, 601 77 Brno, Czech Republic

Inventors: PROKOP, Zbyněk, Palackého tř. 129, 612 00 Brno, Czech Republic, DAMBORSKÝ, Jiří, Bořetická 13, 628 00 Brno, Czech Republic, OPLUŠTIL, František, Synkova 8, 628 00 Brno, Czech Republic, JESENSKÁ, Andrea, Pahrbek 21, Brno 618 00, Czech Republic, NAGATA, Yuji, 2-1-1 Katahira, 980-8577 Sendai, Japan

Contact: Dr. Jiří Damborský, Loschmidt Laboratories
Masaryk University, Faculty of Science,
Kotlarska 2, 611 37 Brno, Czech Republic,
ph 420-5-49493467, fax 420-5-49492556,
e-mail: jiri@chemi.muni.cz

http://loschmidt.chemi.muni.cz/peg
Method of detoxification of yperite by using haloalkane dehalogenases

Field of the invention

This invention relates to method for detoxification of yperite by using haloalkane dehalogenases (Enzyme Commission number EC 3.8.1.5) as a primary, chemically active component of decontamination compositions. Decontamination compositions are designated for detoxification of yperite (2,2'-dichlorodiethylsulfide) on the surface of military hardware, transportational, industrial and agricultural hardware, technical devices and constructional objects (hereafter instrumentation), people’s or animal’s skin and elements of environment (water, soil, sediments and air), that are contaminated by this highly toxic blistering substance.

State of the art

At the present time, there are being used decontamination compositions in the armed forces, civil defense troops, fire services and rescue forces, that exhibit high unit consumption and undesirable aggressiveness on material, because their chemically active components are stechiometric agents, that are gradually consumed during their reaction with yperite. Their application on instrumentation leads to depreciation of decontaminated material or surfaces by corrosion and if these compositions get into soil or water, it endangers environment.

There have been described enzymes in the literature that exhibit activity against highly toxic organophosphorous (neural) substances, called organophosphorous hydrolases, OPA anhydrases or DFPases. As the only example of biological detoxification of blistering yperite (2,2'-dichlorodiethylsulfide) the use of bacteria species \textit{Rhodococcus rhodochrous} IGTS8 (ATCC 53968) was mentioned in the art so far, which has the ability to utilize a chemical analog of yperite 2-chlorethyl-ethylsufite as the only source of carbon for it's growth [Kilbane, J. J., and Jackowski, K. (1996) \textit{J. Chem. Tech. Biotechnol.} 65, 370-374]. Detoxification activity of bacteria species \textit{Rhodococcus rhodochrous} IGTS8 (ATCC 53968) is based on splitting the S-C bond in the molecule. The application of the enzyme splitting C-S bond in a non-toxic product of hydrolysis, thiodiglykol, has been published [Harvey, S., DeFrank, J. J., Valdes, J. J., Kamely, D, and Chakrabarty, A. M., (1990) \textit{Proceedings: Biotechnology-Biodegradation Workshop Symposium by US Army Research Office}, 47-58; Kilbane, J. J., (1990) \textit{Resources Conserv. and Recycl.} 3, 69-79].

Haloalkane dehalogenases are enzymes able to remove halogen from halogenated aliphatic compound by a hydrolytic replacement, forming the corresponding alcohols [Janssen, D. B. Pries, F., and Van der Ploeg, J. R. (1994) \textit{Annual Review of Microbiology} 48, 163-191]. Hydrolytic dehalogenation proceeds by formal nucleophilic substitution of the halogen atom by

**Objects and summary of the invention**

By the method of this invention, the above mentioned imperfections of existing decontamination compositions, that consist of stechiometric agents, are to the great extent overcame by preparations, that consist of catalyst of hydrolytic detoxification of yperite, that is enzyme or mixture of dehaloalkane dehalogenases. The main and significant component of the compositions is the presence of at least one enzyme of the haloalkane dehalogenases. In general this method includes hydrolytic dehalogenation of yperite in the way, that decontamination composition consisting of one or more wild or modified haloalkane dehalogenases affect yperite and convert it to non-toxic product thiodiglycol. Haloalkane dehalogenase is expressed in the natural producer or in a heterologous host organism, e.g. in bacteria *Escherichia coli*, or in yeast *Pichia pastoris*. The enzyme used, can be in non-living or living cells, in the form of crude extract or purified protein. As an enzyme for the dehalogenase composition at least one haloalkane dehalogenase selected from the family of enzymes EC 3.8.1.5 is used, e.g. haloalkane dehalogenase DhaA from *Rhodococcus rhodochrous* NCIMB 13064, DmbA from *Mycobacterium bovis* 5033/66 or LinB from *Sphingomonas paucimobilis* UT26.
Haloalkane dehalogenases constitute an important group of enzymes that are able to cleave the halogen-carbon bond in halogenated aliphatic compounds. They exhibit a broad substrate specificity including haloalkanes, haloalkenes, haloethers and haloalcohols. The mechanism of dehalogenation is based on the nucleophilic attack of the carbon atom to which the halogen is bound and proceeds to cleavage of halogen ion and formation of alkyl-enzyme intermediate. The intermediate is subsequently hydrolyzed with production of corresponding alcohol, halogen ion and proton. The enzyme haloalkane dehalogenase transforms yperite into non-toxic bis(2-hydroxyethyl)sulfide by hydrolytic dehalogenation.

In the decontamination compositions haloalkane dehalogenase can be in crude or purified extract, immobilized on a carrier material, free in aqueous solution, in a monophasic organic or aqueous solution or in organic/aqueous biphasic systems. Enzymes can be immobilized by absorption on the inorganic or organic carrier material (such as: Celite, activated charcoal, aluminium oxide, cellulose, synthetic resins, Sephadex) or covalent attachment onto the surface of organic material (such as: cellulose, dextran, starch, chitin, agarose) inorganic material (such as: porous glass), or synthetic polymeric carrier material (such as: VA-Epoxy Biosyt, Eupergit).

The enzyme haloalkane dehalogenase may be dissolved, crystalline, lyophilized or precipitated. The enzyme can be confined to a restricted area, where it remains catalytically active – entrapped into a solid matrix or into by a membrane restricted compartments. Enzymes may be entrapped into a biological matrix, e.g., agar gel, alginate gel, κ-carragenan. The enzyme can be entrapped also to inorganic stable matrices, e.g., silica gel. A tight network that is able to carry isolated enzyme can be obtained by polymerization of synthetic monomers, e.g., polyacrylamide, in the presence of the enzyme. Depending on the immobilization technique, the properties of the enzyme such as catalytic rate, stability and binding affinity may be significantly altered. The hydrolytic detoxification of yperite catalysed by the enzyme can be performed at the temperature range 10 – 70 ºC with reaction optimum around 40 ºC.

Additional components are aqueous buffer systems (e.g., phosphate buffer, Tris-sulfate buffer, glycine buffer, acetate buffer or citrate buffer) which stabilize the neutral pH being close to optimum interval of 7.0 – 8.5. The pH activity profile is broader and allows pH interval from 4 to 12 while maintaining a reasonable activity. Another additional components are surfactants or organic solvents, that facilitate dissolving /dissolution of yperite in aqueous solvents. Addition of water-miscible organic solvents, e.g., methanol, tert-butanol, aceton, dioxane, acetonitrile, dimethyl formamide, dimethyl sulfoxide, tetrahydrofuran, 3-methyl-3-pentanol and pyridine, can be used at concentration up to 70 % of the total volume depending on the enzyme stability.
Decontamination compositions based on haloalkane dehalogenases can consist of two macroscopic phases, namely the aqueous phase containing the dissolved enzyme and a second phase of organic solvents, partially water soluble or insoluble in water, e.g., ethyl acetate, diethyl ether, methyl tert-butyl ether, cyclohexanol, n-propylacetate, ethyl chloroacetate, bis(2-chloroethyl)ether, isopropyl acetate, butyl acetate, isobutyl acetate, hexanol, isoamyl acetate, n-amyl acetate, toluene, octanol, isoheptane, n-butyl ether, cyclohexane, 2-methylpentane, n-hexane, methylcyclohexane a n-octane. Organic phase enhances solubility of yperite in the decontamination composition which penetrates into water phase. The reaction takes place in aqueous phase, where the enzyme is in natural environment and is not in direct contact with organic solvent, where the most of dissolved yperite is located. The transfer of reactant and product between the two phases, reactant to the enzyme, product from the enzyme, can be increased by enlarging the surface between the two phases (producing a fine dispersion) or by stirring. The bulk water can be replaced by addition of water immiscible organic solvent. The enzyme is than suspended in a monophasic organic solvent. The optimum catalytic activity of the enzyme in organic solvent can be obtained by adjustment and maintenance the water content. This can be conventionally obtained by a pair of salt/hydrate, e.g., CaCl₂ • H₂O/2 H₂O, NaI anh./2 H₂O, Na₂HPO₄ anh./2 H₂O, NaOAc anh./3 H₂O, NaBr anh./2 H₂O, Na₃P₂O₇ anh./7 H₂O, Na₂HPO₄ • 2 H₂O/7 H₂O, Na₂SO₄ anh./10 H₂O, Na₂HPO₄ • 7 H₂O/12 H₂O, that are added to the solvent and function as a water buffer. The enzyme solubility in lipophilic organic solvents can be modified by covalent attachment of the amphiphatic polymer polyethylene glycol to the surface of enzyme. Linkage of the polymer chain onto the enzyme surface is achieved by reaction of ε-amino groups of lysine residues with „linker“, e.g., cyanuric chloride. Protein stabilizers such as polyalcohols, e.g., sugar alcohols or glycerol, inactivated proteins, e.g., bovine serum albumin, or polymers, which show a certain structural resemblance with water, e.g., polyethylene glycol, polyvinyl alcohol, can be added to the reaction medium to enhance the enzyme stability.

Haloalkane dehalogenases used according to this invention can be additionally produced by means of rational design based on structural analysis, e.g., protein crystallography, nuclear magnetic resonance and circular dichroism spectroscopy, and biochemical characterization, e.g., steady-state kinetics, transient kinetics, stability and thermo stability assays, spectroscopic analyses and a like, followed by computer modelling, e.g., sequence comparisons, phylogenetic analysis, homology modelling, molecular docking, molecular mechanics, molecular dynamics, quantum mechanics and multivariate statistics, and DNA mutagenesis, e.g., cassette mutagenesis, site-directed mutagenesis, chemical mutagenesis, error-prone PCR, site saturation mutagenesis, ensemble mutagenesis, recursive ensemble mutagenesis, scanning saturation mutagenesis, mutator strains, etc. The procedure
includes altering at least one amino acid residue of the haloalkane dehalogenase with another amino acid residue or recombining two or more members of the haloalkane dehalogenases to obtain a modified enzyme with improved efficacy. Modified nucleic acids can be introduced into a cell, in which they can be expressed to provide an altered haloalkane dehalogenase.

Advantages of decontamination compositions with haloalkane dehalogenases are, that

a) It posses desired detoxification activity on the yperite, which is homogenously dissolved in aqueous solutions with pH in the range from 4 to 12 (with the neutral optimum of pH 7.0 to 8.5) at the temperature range +0 to +70ºC with reaction optimum around +40 ºC.

b) A low initial concentration (1×10^{-5} mol.l^{-1}) is suitable to attain desired reaction rate, that means a satisfactory level of detoxification in about 15 to 30 minutes.

c) It exhibits catalytic activity, it stays unexhausted during reaction, which brings savings/cost reduction in logistic area.

d) It doesn't exhibit chemical aggressiveness against common construction materials and components of technique that resist the affect of neutral aqueous, corrosively non-aggressive decontamination compositions (aqueous suspensions, foams, emulsions or micro-emulsions).

e) It doesn't exhibit any toxicity and is degradable in environment

Examples of implementation

The enzyme is prepared by homologous expression in native organism/natural producer or by heterologous expression in a host organism, e.g., bacterium *Escherichia coli* or yeast *Pichia pastoris*. According to the invention, the enzyme present in living or non-living cells is used in the form of crude extract or purified protein.

Example 1

To overproduce wild type of enzyme haloalkane dehalogenase LinB from *Sphingomonas paucimobilis* UT26 (Sequence 1) the corresponding gene was cloned in the pPICZαA expressional vector. Cloned plasmids were than transferred into *Pichia pastoris* GS115. *Pichia pastoris* GS115 was than cultured at 28ºC in growth medium (1 weight % of yeast extract, 2 wgt % of peptone, 4×10^{-5} wgt % of Biotine and 1 wgt % of casamino acid in 100 mM potassium phosphate buffer, pH 6.5). The induction of the enzyme synthesis was
initiated by addition of 0.7 volume % of methanol when the culture reached an optical density of 2 at 600 nm. After induction the culture was incubated at 28 °C for 10 h and then harvested. Ammonium sulfate was added to supernatant to a final concentration of 75% of saturation. Solution was stirred 30 min until the added ammonium sulfate was dissolved. The supernatant was centrifuged 15 min at 11 000 g. Pellet was than re-suspended in 20 mM potassium phosphate buffer, pH 7.5 with content of 0.5 M sodium chloride and 10 mM imidazole. The haloalkane dehalogenase was then purified on a Ni-NTA Sepharose column HR 16/10 (Qiagen, Germany). The His-tagged haloalkane dehalogenase was bound to the resin in the equilibrating buffer, which contained 20 mM potassium phosphate buffer pH 7.5; 0.5 M sodium chloride and 10 mM imidazole. Unbound and weekly bound proteins were washed off by buffer containing 60 mM imidazole. Then the His-tagged enzyme LinB was eluted by buffer containing 160 mM imidazole. The active fractions were dialyzed overnight against 50 mM potassium phosphate buffer, pH = 7.5. The enzyme was stored at 4 °C in 50 mM potassium phosphate buffer, pH 7.5, containing 10 % glycerol and 1 mM 2-mercaptoethanol to enhance long-lasting enzyme stability.

![Graph showing conversion of yperite](image)

**Fig. 1** – Conversion of yperite by the use of haloalkane dehalogenase LinB. Spontaneous hydrolysis of yperite without enzyme $k_c = 0.0046$ s$^{-1}$ (empty circles) and degradation of yperite in the presence of haloalkane dehalogenase LinB $k_{cat}/K_m = 6.9$ s$^{-1}$.mM$^{-1}$ (black/filled circles).
Hydrolytic dehalogenation catalyzed by haloalkane dehalogenase (wild type or modified) converts the toxic yperite into non-toxic bis(2-hydroxyethyl)sulfite. The hydrolytic dehalogenation of yperite was catalyzed by haloalkane dehalogenase at 37 °C in 50 mM phosphate buffer (pH 7.5; adjusted by addition of 1M NaOH solution). The yperite was added into reaction buffer to its final concentration in buffer of 94.3 mg.l⁻¹ of yperite. Reaction was initiated by addition of solution of enzyme LinB (50 mM potassium phosphate buffer pH 7.5; 1×10⁻⁵ to 1×10⁻⁴ mol.l⁻¹ of haloalkane dehalogenase LinB, 10 vol % of glycerol and 1 mmol.l⁻¹ 2-mercaptoethanol). Operation of haloalkane dehalogenase leads to rapid and complete decontamination of the yperite. The kinetics of reaction is shown in Figure 1. Catalytic activity/power of haloalkane dehalogenase LinB by decontamination of yperite is \( k_{\text{cat}}/K_m = 6.9 \text{s}^{-1}\text{mM}^{-1}\).

Example 2

To overproduce enzyme haloalkane dehalogenase DhaA from *Rhodococcus rhodochrous* NCIMB 13064 (Sequence 2), the corresponding gene was cloned in the pAQN vector containing *tac* promotor (P\text{tac}) under the control of *lacI*. *Escherichia coli* BL21 containing pAQN plasmid was cultured in 250 ml Luria broth at 37 °C. The induction of enzyme synthesis was initiated by addition of isopropyl-β-D-thiogalactopyranoside to a final concentration of 0.5 mM when the culture reached an optical density of 0.6 at 600 nm. After induction, the culture was incubated at 30°C for 4 h and then harvested. The cells were disrupted by sonication using a Soniprep 150 (Sanyo, UK). The supernatant was used after centrifugation at 20000 g for 1 h. The haloalkane dehalogenase was purified on a Ni-NTA Sepharose column HR 16/10 (Qiagen, Germany). His-tagged haloalkane dehalogenase was bound onto the resin in the equilibrating buffer, which contained 20 mM potassium phosphate buffer pH 7.5, 0.5 M sodium chloride and 10 mM imidazole. Unbound and weakly bound proteins were washed off by buffer containing 60 mM imidazole. The His-tagged haloalkane dehalogenase was then eluted by buffer containing 160 mM imidazole. The active fractions were dialysed overnight against 50 mM potassium phosphate buffer, pH 7.5. The enzyme was stored at 4 °C in 50 mM potassium phosphate buffer, pH 7.5, containing 10 % glycerol and 1mM 2-mercaptoethanol enhancing long-lasting enzyme stability.

Enzyme haloalkane dehalogenase DhaA or its variant is the part of decontamination composition that contains aforementioned enzyme at concentration $1 \times 10^{-6}$ to $1 \times 10^{-4}$ mol.l$^{-1}$, further 1 to 5 vol % of aliphatic hydrocarbon of general formula C$_n$H$_{2n+2}$ or cyclic aliphatic hydrocarbon of general formula C$_n$H$_{2n}$, where n is 6 to 12, further 5 to 20 vol % of aliphatic alcohol of general formula C$_n$H$_{2n+1}$OH, where n is 2 to 4, further 3 to 15 wgt % of anion active tenside of general formula C$_n$H$_{2n+1}$OSO$_3$Me, where n is 10 to 16 and Me stands for counter ion (Na$^+$, K$^+$ or monoethanol amonium), 1 to 10 wgt % alkylbenzensulfonate of general formula R$^{(3)}$-(Ar)SO$_3$-Me$^+$, where R$^{(3)}$ stands for alkyl with 11 to 13 atoms of carbon, and Me$^+$ indicates sodium ion, further components of glycine buffer to adjust pH of aqueous solution in the range from 7 to 9, or else glycine of total concentration 0.1 mol.l$^{-1}$, etc. Required pH 8.2 is reached by addition of 1M NaOH. The rest to make 100% is water. The catalytic power of haloalkane dehalogenase DhaA at decontamination of yperite is $k_{cat}/K_m = 5.7$ s$^{-1}$.mM$^{-1}$. 
Sequence 2. Sequence of the gene *dhaA* and haloalkane dehalogenase DhaA isolated from bacterium *Rhodococcus rhodochrous* NCIMB 13064.

```
atg tca gaa atc ggt aca ggc ttc ccc ttc gac ccc cat tat gta gaa gtc ctg ggc gag cgt atg cac tac
gtc gat tgt gga ccc ccg gat ggc acg cct gtg ttc ctg gag ctc aac cgg acc tgg gcc ttc tac tgc cgc
acc ctc ccg gat gta cca gcc aag cgc aat ccg cga cgg gcc aaa ggt att gca tgt atg gaa ttc atc cgg cct
agt ggc gac ctg gtc cgg ccc cat gta gca cgg agg cat cgg tgc att gct cca gac ctg atc ggg atg gga aac
tcg gaa cca gac ctc gat tat ttc ttc gac gac cac gtc cgg cgc tac ctc gat gtc ccc ttc atc gaa ggc ttg
gtg gaa gag gtc gtc ctg gtc atc cac gac tgg ggc tca gct ctc gta ttc cac tgg gcc aag cgc aat ccg cga
cgg gtc aat gtt gta gaa ttc atc cgg cct atc cgg acg tgg gac gaa tgg ccg gaa ttc gcc cgt gag acc
ttc cag gcc ctc cgg acc gcc gag gtc ggc cga cgg ctt gac gtt ctc cgg ccc ctt gtc gat gtt ggt gcc
ctc aag cct gtt gac gga gaa tgg cta gca ctt ggc ttc gcc ccc aac gac ctg tgc ccc ctc gcc ggt gag
cgg cgc atc gtc cgg ccc ctt ggt gac gtc tgg ggc cca ctc gcc gca cgg gcc gaa gcc ggc aga ctt gcc
gaa ggc ccc ttc gcc acc tgc aag cca atc gcc cgg cga ttc cac tgc cag gaa gac aac ctg gtc gtc atc
ctg gtc gta atc gcc cgg cca ctc gcc gag cct atc gcc ggt gag ccc gcg aac ttc cct ctc ctc cag
MSEIGTGFPFDHYVEVLGEMRMYVDDGPRDGTPVFLHGNPTSSYLWNIELKHVAPSHRCIA
PDLIMGKDKDYYFDDHVRLDFAIEALGEEVVLVIHDWGSALGFHWAKRNPERVKGID
ACMEFIRPIPTWDEWPFARFQAFRTADVGRELIDQNAFLGAEALPKCVVRPLTEVEMDHYR
EPFLKPVDREPLWRFPNELPIAGEPANIVALVEAYMNWLHTLSPVPLLFWGTGVLIPPAEAA
RLAESLPNCRTVDIGPGLHYLQEDNPDLIGSEIARWLPAL
```

Example 3

To overproduce enzyme haloalkane dehalogenase DmbA from *Mycobacterium bovis* 5033/66 (Sequence 3), the same procedure as in example 1 is followed. Enzyme haloalkane dehalogenase DmbA or its variant is the part of decontamination composition that contains aforementioned enzyme at concentrations $1 \times 10^{-6}$ to $1 \times 10^{-4}$ mol.l$^{-1}$, further 1 to 20 vol % of aliphatic alcohol of general formula $\text{C}_n\text{H}_{2n+1}\text{OH}$, where $n$ is 2 to 4, further 3 to 15 wgt % of anion active surfactant of general formula $\text{C}_n\text{H}_{2n+1}\text{OSO}_3\text{Me}$, where $n$ is 10 to 16 and Me stands for counter ion ($\text{Na}^+$, $\text{K}^+$ or monoethanol amonium), 1 to 10 wgt % of ethoxy nonylphenol of general formula $\text{C}_9\text{H}_{19}-\text{Ar-O-(C}_2\text{H}_4\text{O})_n\text{H}$, where $n$ is 9 to 10, further components of glycine buffer to adjust pH of aqueous solution in the range from 7 to 9, or else glycine of total concentration 0.1 mol.l$^{-1}$, etc. Required pH is reached by addition of 1M NaOH. The rest to
make 100% is water. The catalytic power of haloalkane dehalogenase DmbA at
decontamination of yperite is \( k_{cat}/k_m = 6.0 \text{ s}^{-1}.\text{mM}^{-1} \).

Sequence 3. Sequence of the gene \textit{dmbA} and haloalkane dehalogenase DmbA isolated
from bacterium \textit{Mycobacterium bovis} 5033/66.

```
ATG ACA GCA TTC GGC GTC GAG CCC TTC GGG CAG CCG AAG AAC ATG GCA
TAT ATC GAC GAA GGC AAG GGT GAC GCC ATC TTT CAG CAC GAG AAC CAC
ACC AGC CCA TCG GGA CCC GAG GCT GCG GGC ATC CAG CAC TGG GTG
```

Example 4

Enzyme haloalkane dehalogenase LinB or its variant is the part of decontamination
composition that contains the enzyme at concentrations 1×10\(^{-6}\) to 1×10\(^{-4}\) mol.l\(^{-1}\), further 1 to 15 wgt % of anion active surfactant of general formula \( \text{C}_n\text{H}_{2n+1}\text{OSO}_3\text{Me} \), where \( n \) is 10 to 16 and Me stands for protion (\( \text{Na}^+ \), \( \text{K}^+ \) or monoethanol amonium), 1 to 10 wgt % of ethoxylated nonylphenol of general formula \( \text{C}_9\text{H}_{19}-\text{Ar}-\text{O}-(\text{C}_2\text{H}_4\text{O})_n\text{H} \), where \( n \) is 9 to 10, further components of phosphate buffer to adjust pH of aqueous solution in the range from 7 to 8.5; that is \( \text{KH}_2\text{PO}_4 \) a \( \text{K}_2\text{HPO}_4 \) in the required ratio and in a total concentration 50 mmol.l\(^{-1}\), etc. The rest to make
100% is water.
Industrial utility

This invention is utilized in industry to eliminate yperite from the surfaces of military hardware, transportational, industrial and agricultural hardware, technical devices and constructional objects, of the people’s or animal’s skin and elements of environment, that are contaminated by this highly toxic blistering substance. This technology is utilized in armed forces and also in civil services, generally there, where is need to use decontamination compositions to decontaminate blistering substances.
CLAIMS

1. The method of detoxification of yperite by the use of haloalkane dehalogenases, determined by that, that yperite is exposed to at least one haloalkane dehalogenase selected from the group of enzymes with EC 3.8.1.5, with concentration of enzyme $1 \times 10^{-6}$ to $1 \times 10^{0}$ mol.l$^{-1}$, at temperature from $+10 \, ^{\circ}C$ to $+70 \, ^{\circ}C$ and pH = 4 to 12 in liquid medium.

2. The method according to claim 1, determined by that, that haloalkane dehalogenase is an enzyme selected from the group containing dehalogenase DhaA from *Rhodococcus rodochrous* NCIMB 13064, DmbA from *Mycobacterium bovis* 5033/66, LinB from *Sphingomonas paucimobilis* UT26.

3. The method according to claim 1 and 2, determined by that, that used haloalkane dehalogenase is wild type or modified haloalkane dehalogenase or their mixture.

4. The method according to claims 1 to 3, determined by that, that the preparation of haloalkane dehalogenase is carried out in the presence of at least one protein stabilizer, selected from the group containing polyalcohols, inactive proteins, or polymers.

5. The method according to claims 1 to 4, determined by that, that the liquid medium is an organic solvent, a mono-phasic aqueous solution of an organic solvent, a biphasic system of organic and aqueous components.

6. The method according to claims 1 to 5, determined by that, that enzyme haloalkane dehalogenase is used in soluble form or crystalline or lyophilized or precipitated form.

7. The method of detoxification of yperite according to claims 1 to 6, determined by that, that enzyme haloalkane dehalogenase is immobilized by absorption or by ionic binding or by covalent attachment onto the surface of a carrier.

8. The method of detoxification of yperite according to claims 1 to 6, determined by that, that enzyme haloalkane dehalogenase is immobilized by cross-linking (linkage to each other) or entrapping enzyme into a solid matrix or a compartment confined by a membrane.

9. The method according to claims 1 to 8, determined by that, that detoxification is carried out in the presence of surfactants.
Abstract

Title: Method of detoxification of yperite by using haloalkane dehalogenases

The method of detoxification of yperite by the use of haloalkane dehalogenases or their compositions, the method of preparation of dehalogenationating enzymes and of decontamination compositions which contain at least one wild type and/or modified haloalkane dehalogenase (EC 3.8.1.5) as an chemically active component. The preferred dehalogenases are DhaA from *Rhodococcus rhodochrous* NCIMB 13064, DmbA from *Mycobacterium bovis* 5033/66 or LinB from *Sphingomonas paucimobilis* UT26. Decontamination is utilized for detoxification of yperite from the surfaces of instrumentality, constructional objects, the people’s or animal’s skin and elements of environment, when yperite is exposed to the action of decontamination composition at +10 °C to +70 °C, preferably at +40 °C and pH = 4 to 12.