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## **INTRODUCTION**

#### 1. The pollution-management system invention

#### 1.1 The subject of the base invention

The subject of the base invention is a pollution-management system whose common characteristic is the biodegradation<sup>1</sup> of organic contaminations with enzyme<sup>2</sup> systems produced by specially selected microorganisms.

In the course of biodegradation – with the presence of mobilized nitrogen, phosphor and potassium – the enzymes produced by the microorganisms transform the hydrocarbons into fatty acids which produce carbon dioxide and water. In the pollution management technology, enzymes extracted from living organisms are allocated directly to the contaminated fluid, so the degradation of the pollution is not done by the microorganisms living in the contaminated area but by the enzyme system. The application of the technology may report the following benefits:

- $\mathfrak{R}$  Fast reaction time of the enzyme-catalyzed processes;
- R The enzyme systems in contrast to bacteria are not sensitive to toxic compounds;
- The enzyme systems are not sensitive to the demolished compound concentrations of hydrocarbons;
- $\mathfrak{R}$  Nutrient dosage is not necessary;
- $\mathfrak{A}$  Aquatic environment use;
- R Turns dirt into ecologically recyclable natural materials;
- ℵ Non-poisoning, there is no irritant effect on the human body (skin, eyes, respiratory system);
- $\mathfrak{R}$  Using protective clothing is not required.

The common principle of the pollution-management system is the biochemical, enzymatic transformation of contaminations into environment-friendly products, occasionally suitable for further use. The enzyme preparation consists of enzymes produced by microorganisms and living, specially selected strains of bacteria. The preparation performs the disintegration of pollutions taking place in nature concentrated, as it was "self-catalyzed". Its application eliminates the disadvantages of the traditional management systems, not only by protecting the environment, but also by benefitting the further biochemical processes of the contaminated area (ground, water).

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2. Enzyme – catalyst of biochemical processes

<sup>1.</sup> Biodegradation – the controlled biological disintegration of hydrocarbon contaminations. The biodegradation grants therefore in some sort the decay, the disposal of toxic contaminations and the termination of the damaging effect, usually in the course of multiple-step degrading processes.

### TECHNOLOGY

#### **1.2 Technological characteristics, working principle**

The common base technology of the systems to treat the pollution is spraying the specially coded enzyme culture into the water or the ground, or working it into the soil in the given area. This so called active material conducts the biochemical transformation of environmental pollutants in a single phase. The enzyme preparation base system with professional innovation can be adapted and further developed to almost any type of environmental pollution. The enzyme preparation contains proteins produced by living organisms. The action of the enzymes is specific as regards both the degradable substances and the circumstances under which they can function efficiently. However, various substances require specific enzymes. Therefore, the enzyme preparation contains inter alia the four most important types of enzymes:

- $\mathfrak{R}$  Lipase to break down oils and fats;
- R Amylase to break down carbohydrates and starches;
- $\mathfrak{R}$  Protease to break down proteins;
- $\mathfrak{R}$  Cellulase to break down vegetable fibre.

For the stability of the enzyme preparation and to broaden its impact, the mixing of multiple enzymes in order and at the appropriate temperature was necessary. The selection of the bacteria was done on the basis of producing such enzymes as broadly as possible which are able to disintegrate and handle various contaminations within a wide range of temperature and pH limits. The strains of bacteria acts essentially like 'enzyme factories' in the preparation. Given that the pollutions, the polluted areas are rarely homogeneous, the pollution concentration is very variable; the required quantity for treatment of the various types of contamination has to be exactly defined with an on-the-spot test examination. The specially selected enzyme system speeds up the naturally also occurring degradation processes by disintegrating the chemical bonds of the hydrocarbon pollutions due to the catalyzing effect of the enzymes immeasurably.

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The products of the enzyme system family are made up of specific enzyme systems, adapted to the biodegradation of various pollutions. These enzyme systems are efficient under aerobe circumstances. Their appliance eliminates the disadvantages of the traditional management systems, not only by protecting the environment, but also by benefitting the further biochemical processes of the contaminated area (ground, water).

Another common characteristic of the enzyme system family is that it is highly dilutable. The type of the used enzyme system and the concentration of dilution depend on the amount of pollutant emissions in the environment, the spread of the contamination, the affected elements of environment, the characteristics of the spread, the exposure routes connected with the use of the location and on the harmful impact of the pollutant.

#### 1.3 The significance of the invention and its positioning

The significance of the enzyme preparation as invention lies in its potential uses and in the results following its use and application. The invention is essentially a special combination of enzymes and living strains of bacteria, which provides enhanced variations responding to the needs of use. Its range of effects and adaptability are extensive, its possibilities of further development coded in the base invention are almost unlimited. The significance of the enzyme preparation developed for pollution-management can be characterized as follows:

- $\Im$  In scientific terms it is the discovery of the enzyme preparation and the proper practical use of its qualities;
- $\Im$  In technical terms it has environment-friendly mechanism of action; it transforms the disintegration of environmental contaminations into an environment-friendly product, occasionally suitable for further use;
- $\mathfrak{R}$  In technological terms it is a system adapted to different pollutions that can be further extended with proper innovation to handle other pollutions;
- ℜ In economic terms it is a cost-saving pollution-management procedure that does not require high investment and can be carried out on location in a single phase mechanism of action without further materials and technologies.

In summary, the enzyme preparations create a system family that are suitable for most frequent treatment, in a way that both the enzyme preparations and the due to the effect of the enzymes transformed substances are environment-friendly products.

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#### 2. Application opportunities

#### 2.1 Pollution types that maybe treated with the living-culture enzyme preparation

The enzyme preparation can be used for treating the following pollutions:

- ♥ various types of oils (gasoline, heavy oil, silicon oils);
- R oil derivatives;
- R petrol, kerosene;
- R phenols;
- R PCB;
- R DDT;
- R Carboxylic acids.

#### 2.2. Main application areas

- $\mathfrak{R}$  in the upper and deeper levels of the soil;
- R in artificial fairings, on artificial fairings (asphalt, concrete);
- $\mathfrak{R}$  in natural environments, mines;
- at outer and inner cleaning of tankers, tank cars, oil tankers (no special workhouse or hall needed);
- R On water surface, in deep waters and karstic waters.

#### **3. Benefits of the use of the enzyme preparation**

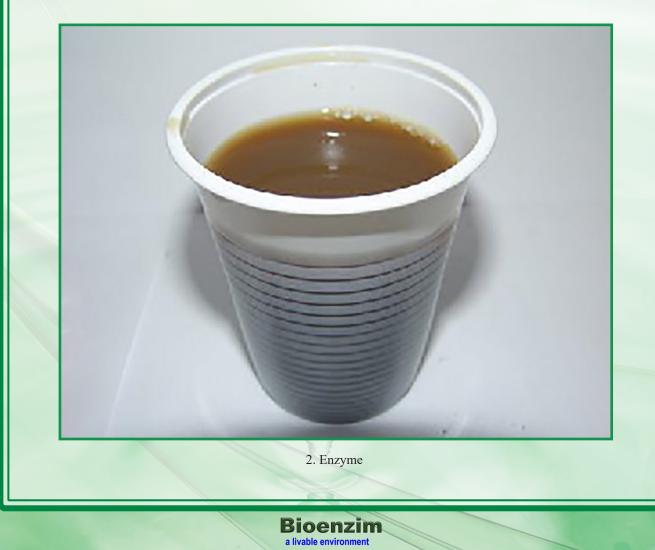
- $\aleph$  It can be applied right on the location where the pollution has had formed;
- $\aleph$  It is suitable for the decontamination of both aboveground and deeper layers of ground and waters;
- $\mathfrak{R}$  The costs of application are smaller in comparison to the generally known systems;
- $\mathfrak{R}$  The pollution-management itself concludes in a short amount of time;
- $\mathfrak{R}$  It can transform the pollutants fully and effectively;
- R The product emerging during the biochemical transformation of the pollutants can be left on location, it is environment-friendly and it has no harmful effects on living organisms as justified by laboratory tests.

The enzyme preparation starts to have its effect in 10 to 40 days, over 10 °C, depending on the concentration and age of the pollution and on the structure of the polluted agent (e.g. type of soil, clay, sandy etc.). The outcomes of the pollution-management are partly carbon dioxide, partly carbonic acid. Regarding to the fact that the polluted agent (primarily the soil) does neither need to be transported nor moved on location (to be digested, dug etc.), the application of the technology characterized above may be with min. 25% savings cheaper compared to the price of the presently applied waste-management systems.





1. Laboratory-test of contamination-handling by enzyme





3. Ground sample before adding oil



4. Ground contaminated by oil before adding enzyme



5. Adding enzyme to oil contaminated ground





6. Oil-contaminated ground worked by Living-culture enzyme preparation 3rd day.



7. Oil-contaminated ground worked by Living-culture enzyme preparation 10th day



8. Oil-contaminated ground worked by Living-culture enzyme preparation 14th day







making of the holes for treatment

installation of perforated pleated hoses



enzyme in the treatment holes



fill-up of the holes with enzyme





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polluted wagon



cleaned wagon

# Algae disposal system

The object of the innovation is the algae disposal enzyme preparation. It has been created as a biological enzyme group in a special laboratory, to which in the proper order and temperature range – with a dedicated technology – the living-cultures are added which ensure the product to reach the wished mechanism of action. The compounds are defined by the results of the actual tests before the applications.

#### **Application technology:**

- $\aleph$  the active substance can be applied with simple sprayers onto the algae;
- $\mathfrak{R}$  the active substance should be applied on the area with high-pressure sprayers ;
- A after 1-2 days, where the browning and sinking of the algae has not started yet, the spraying must be repeated;
- R the browned algae could be picked up and used as e.g. compost or needs to be left sinking down, where the ultimate decay takes place.

#### **Mechanism of action:**

- $\aleph$  ideal temperature for application between +10 °C and +30 °C;
- the application starts with setting of the concentration depending on the amount of algae, 1 litre of active substance can be diluted by its tenfold;
- A at the first phase, it eliminates the algae (the algae browns, the chlorophyll pigments cannot fulfil their purpose);
- A at the second phase, the algae lose their ability to float on the water surface and they slowly sink down, where due to the loss of total light the eliminating process concludes;
- R During the slow sinking the killed algae can provide a feeding ground to higher organisms, primarily to freshwater snails;
- $\aleph$  After that, due to the effect of the enzyme supplement, the algae decays into utilized elements for the microorganisms of the lake.

#### **Efficiency:**

 $\Im$  100 %, regarding that the product can be sprayed again immediately,

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♥ On parts of the area that need a repeated spraying, it can be diagnosed in 1-2 days with survey.

#### Storage temperature and circumstances:

- $\mathfrak{R}$  2-5 °C ca. 6 months;
- R Storage: in sealed, chemical effect-resisting containers.

#### **Characteristics:**

- **Quick effect**, depending on outer temperature (5-7 days at 15-35 °C water temperature);
- R Does not overthrow the biological balance of the lake water;
- The killed amount of algae provides food for both higher functioning (snails) and lower functioning creatures;
- $\mathfrak{R}$  Its application does not come with chemical pollution;
- $\mathfrak{R}$  Its application does not come with oxygen deprivation;
- $\mathfrak{R}$  It not only prevents the algae from growing but eliminates them;
- $\mathfrak{R}$  It not only prevents the algae from growing but eliminates them;
- According to special needs, it can be re-used in the agriculture. The removed and eliminated algae is excellent for application after composting;
- The active substance takes its disintegrating effect only on the algae and other organic lifeless contaminations;
- $\mathfrak{A}$  Environment-friendly;
- $\mathfrak{R}$  It does not require high investment;
- $\mathfrak{R}$  It does not require any other after-treatment.

#### Spraying of the active substance:

R Depending on the technique of getting the substance on the water surface: from manual labour force spraying technique to helicopter spraying technique there are many possible variations, according to the agreement with the Customer.

By the application of our algae disposal technology it should be taken into account that our product is ought to be used both as a preventive treatment and in the early stages of algal bloom.

#### **Reference videos:**

https://www.youtube.com/watch?v=17yI\_uADgdc https://www.youtube.com/watch?v=83IIaOikcNM https://www.youtube.com/watch?v=vFCaIC5lLqI&t=25s&spfreload=10



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# **CHEMICAL DECONTAMINATION AND RECULTIVATION**





Contaminated sand samples by strong chemicals from Libya before treatment. There was attempts but failed implantations of seeds, there is no living organism.



After treatment of different cultures of Ensynox enzyme there is results. Seeds of grass has shown life in the contaminated sand and was going to be grown.

#### **Discussions:**

Enzynox has shown efficiency in case of treat the ground / soil / sand and brings the life into the contaminated soil.

#### Under treatment of Ensynox enzyme 4-10 days later



# ORIGINAL MICROLAB Ltd., TESTING LABORATORY

Executor test :

Company accredited to ISO/IEC 17025 : 2005, General requirements for the Competence of testing and calibration laboratories.

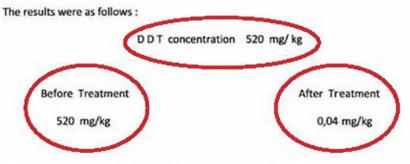
Customer test :

We hereby issue the following statement for the Company Enzimexpo Ltd.

Certificate no, 3605531, the product of the Enzimexpo Ltd. is obtained from specially selected earthworms, "Ensen Foetidia", and those enzymes are capable of cleaning a wide range of contamination products and also able to interfere in different chemical chains.

Sample : 3 x 0,10 kg soil allegedly from Mongolia ,DDT contaminated.

Method : As analytical methods for carbohydrates degradation estimation gas chromatography UV and IR spectrophometry were used.



Result : Degradation enzyma of PCB, DDT, pesticides, ascarel et cetera ...

Degradation of PCB,DDT is very useful because it first step is going through the phenol way and the second as PCB. Pesticides are containing the similar compounds as a previous group and therefore are split into CO2, water, chlorides, or phosphates, which are very quickly bound to sodium in a soil and by such a wary are neutralised. However the need of phosphates in a soil is very well known an the contet of carbon dioxide for nitrification bacteria too.

Rimavská Sobota 26.11.2014

Authorized officer... MVDr.Janka Krišková





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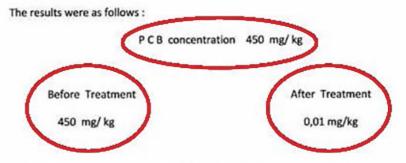
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