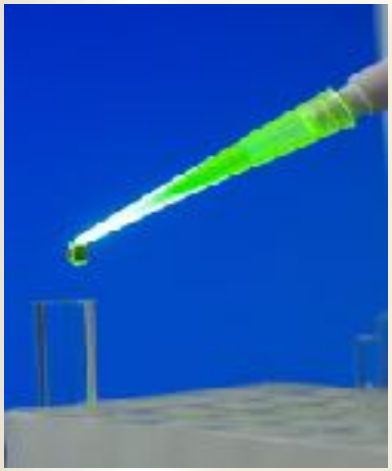

BIOPHYSICAL APPROACHES IN ENZYMATIC BIOASSAYS

From idea to commercial products.

***Problems and advantages*.**

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Problems

- **A growing need for RAPID TOXICITY SCREENING TESTS**
  - ***Environment***: rapid detection of potentially toxic agents (more 25000) in water, soil and atmosphere.
  - ***Healthcare***: medical diagnostics, blood level monitoring of therapeutic drugs etc.
  - ***Food safety***: screening for food borne illnesses
- **SIMPLICITY-PRACTIICALLY -COSTS**

http://www.artsides.ru/

THE TOXICITY SCREENING TESTS

- ***Chemical analysis*** shows which known pollutants in what concentrations are contaminated the sample in comparison with the MPC.
- ***Living organisms toxicity tests*** show the **effect** of harmful substances **on the function of living organisms** (fish, algae, plant, Daphnia, bacteria etc.)

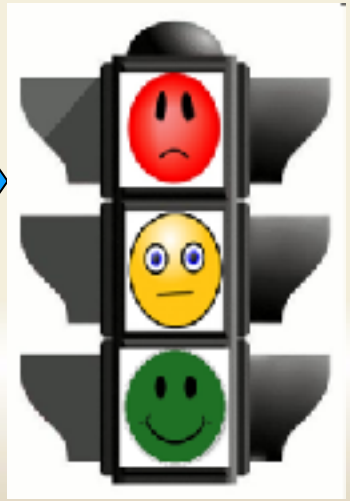
Toxicity bioassay: aim?

The aim is to attract attention to an extraordinary situation and to do this quickly and in the simplest and cheapest way

Rapid detection system of appearance of toxic compounds in environment

Screen for Toxicity

Identification of toxicants

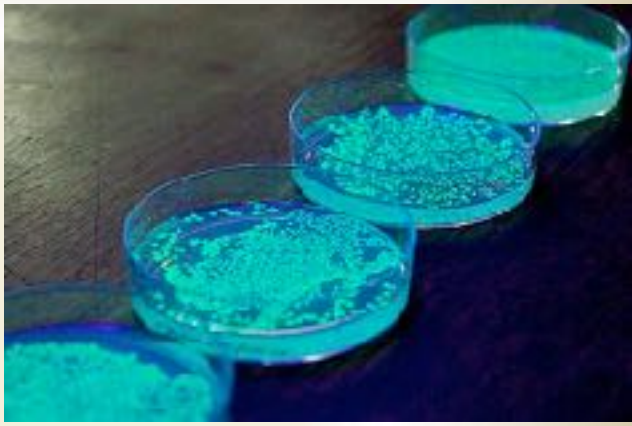


Chemical analysis

Limitations of the current toxicity bioassays

- No be feasible "**in house**", no **routine**, no **practical**,
- no **user-friendly** (need trained personnel for complex performance technology), culturing of live stocks, lengthy preparatory steps,
- **expensive** in equipment and materials,
- no **rapid** results,
- high measurement **error**, low **repeatability**,
- used only for environmental monitoring,
- no **kit** for analysis of a large number of samples.

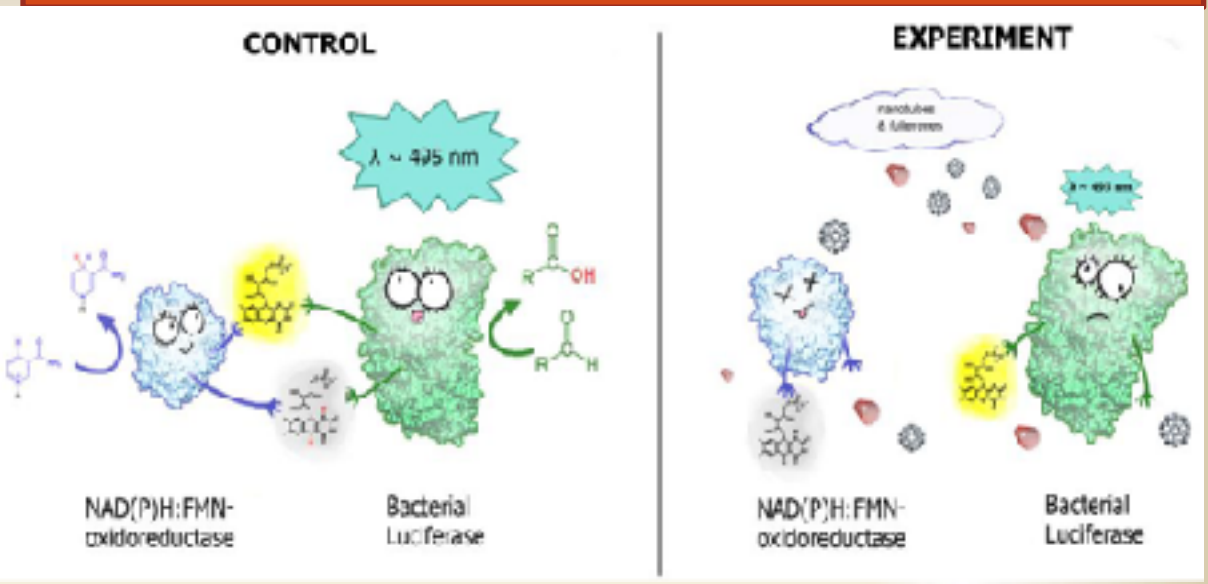
Luminous bacteria toxicity tests: disadvantages



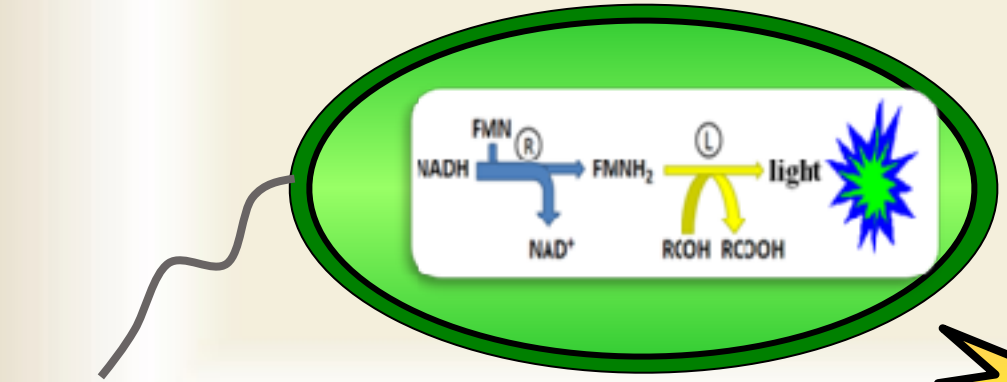
- to maintain the stable bacterial culture during measurements
- low accuracy of measurement
- effect of the toxic substances either by decreasing or by increasing the luminous intensity

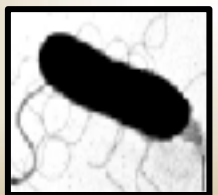
So, the luminous bacteria assay didn't show reliable results.

Idea (V. Kratasyuk, 1985)



From luminous bacteria to enzymes?





hν

490

HM

Luminous bacteria
NADH:FMN-oxidoreductase+luciferase

Scheme of Analysis

Luminous bacteria, Enzymes

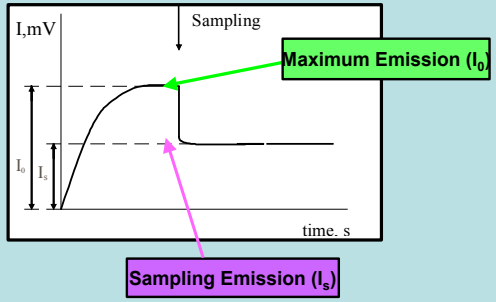
control → luminescence I_c

test → luminescence I_e

sample

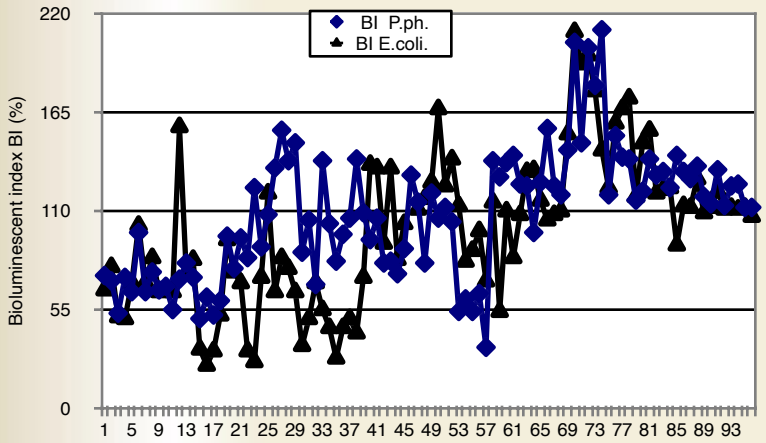
Bioluminometer

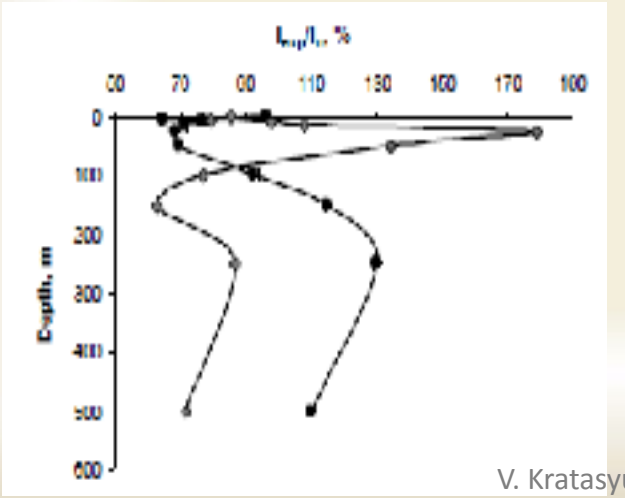
Bacterial Index ($BI = I_s / I_0$)

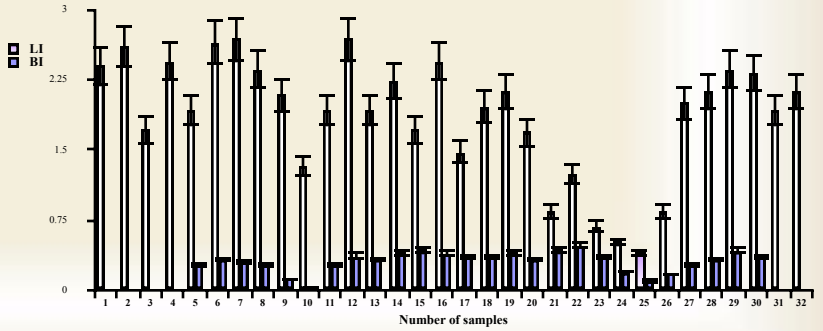


Luciferase Indexes ($LI = I_s / I_0$)

The cost-effective bioluminescence-based toxicity enzymatic bioassay for **environmental control** of natural ecosystems and industry wastewaters, soil and air quality







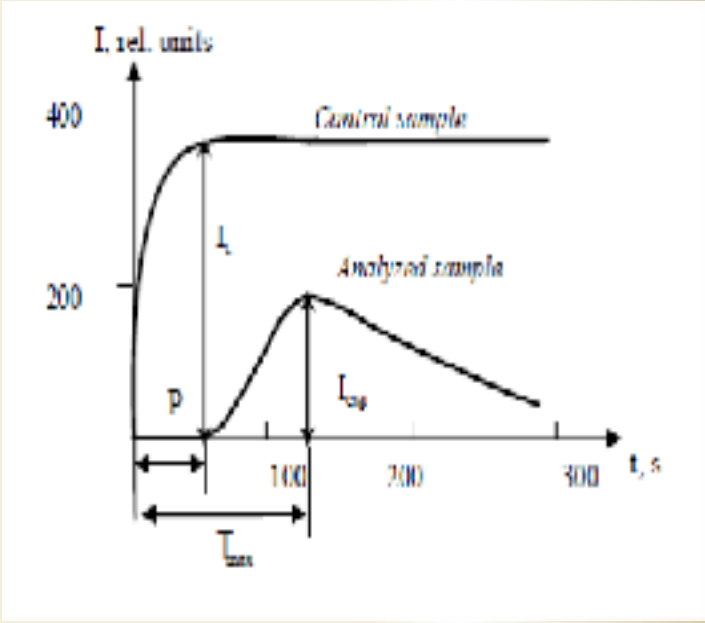
Bioluminescent enzyme system technology BEST Designer Bioassays

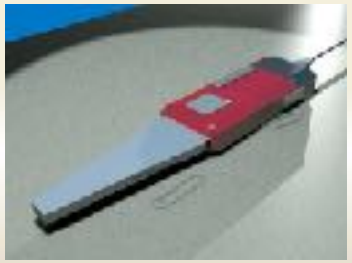
The advantages

Select BioLum system sensitive to specific analyte or sum of toxicants → ***Specificity Versatility***

Optimize the composition → ***Sensitivity***

Immobilized reagents → ***Stability, Rapidity, One-step detection***





- Time of analysis – 1-3 minutes.

The bioluminescent laboratory “Enzymolum”

➢**Reagent “Enzymolum”**
yield of enzymes activity 40 - 50 %

➢ temperature of storage 0^0C + 25^0C

➢ Shelf life > 3 year

➢Desigh of composition

- **Portable bioluminometer “LumiShort”**
- **Bioluminescent enzyme toxicity bioassay**

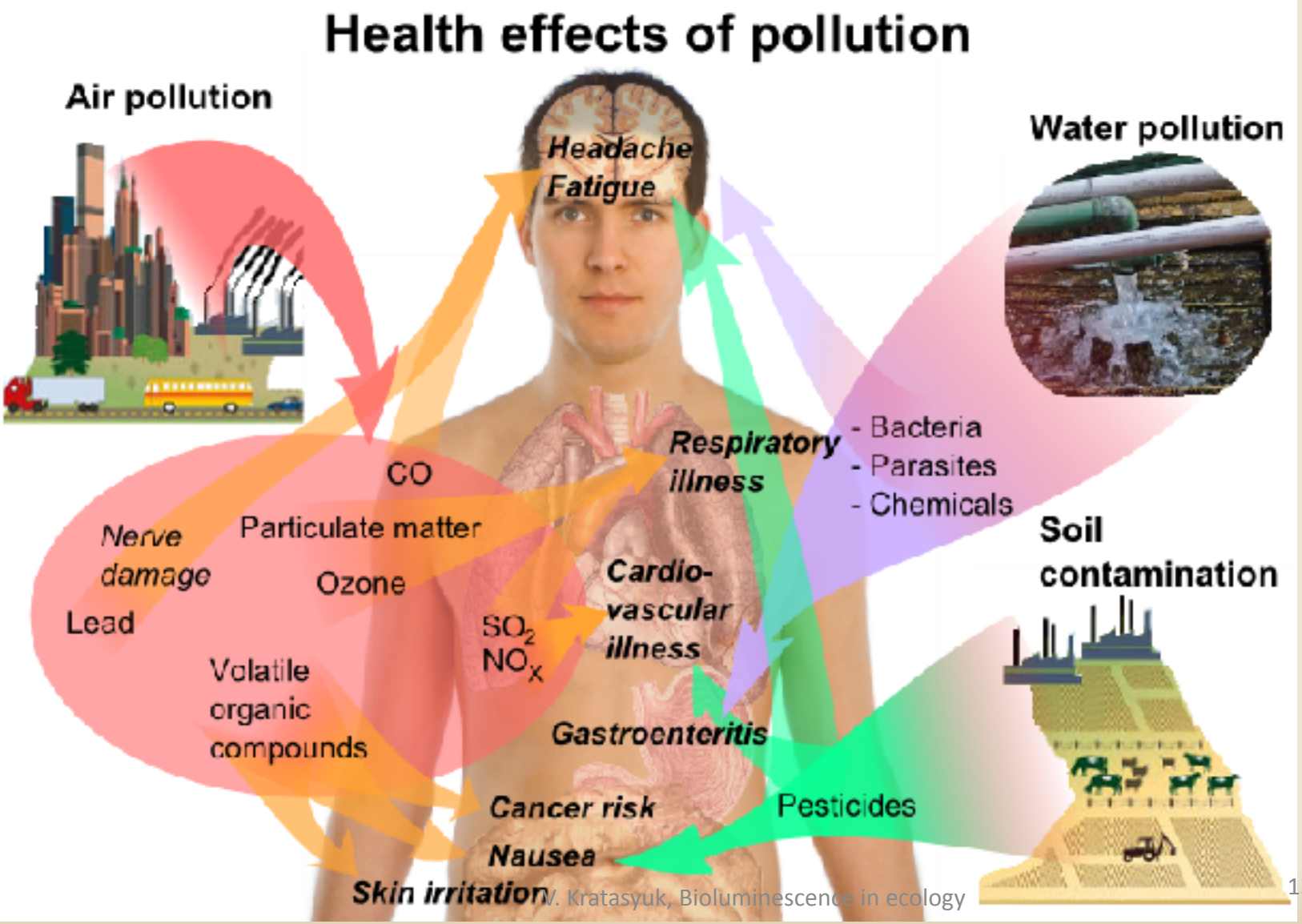
BEST: Bioluminescent Enzyme System Technology

*Cheaper … **Faster** … Better*

	Cost per test	Time to result	Sensi-tivity	Repea-tability of results	Person-nel	Sample prepa-ration	Storage / shipping handling	Rea-gent toxicity
Bio Tests	$380 - $3,240	up to 7 days	Stan-dard	Low	High level train-ing	Require d	Living organism culture, +15 to +30	Non-toxic
BEST™	**$10 - $100**	**Ins-tant result**	**Cus-tom**	**Very high**	**Low level of trai-ning**	**Not needed**	**-30C to +50C**	**Non-toxic**

- Highly adaptable proprietary, protected platform technology
  - Rapid/emergency screening adaptability
  - Shelf-life of reagents up to several years
  - Broad screening – 25,000 toxins under organic compounds
  - Compatible with standard laboratory equipment
  - The biological part of biosensor

Is there a correlation between the results of bioassays and the influence of toxic substances on living organisms ?



The connection between the activity of enzymes and living functions

NADH-oxidoreductase	⇨	Energy metabolism and respiration
Lactate dehydrogenase	⇨	Glucose catabolism for energy synthesis
Trypsin Chymotrypsin	⇨	Hydrolysis proteins and peptides in food
Alcohol dehydrogenases	⇨	Interconversion between alcohols and aldehydes
Butyryl choline esterase	⇨	Control of neurotransmitter activity in the CNS

The enzymatic model of organism as new complex enzymatic toxicity bioassay

Lactate dehydrogenase

Alcohol dehydrogenases

Polyphenol oxidase

Pepsin

Isocitrate dehydrogenase

Glycerol dehydrogenase

Glycogen phosphorylase

Phosphokinase

Glycogen phosphorylase

Hexokinase

Fumarase

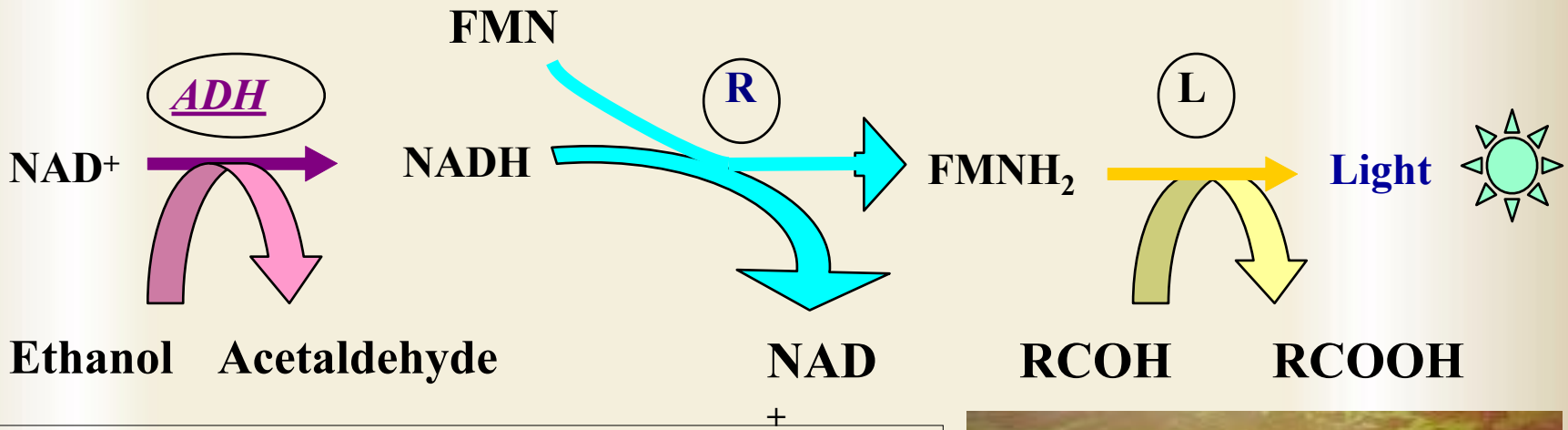
Monoamine oxidase

Malate dehydrogenase

Glucose-6-phosphate dehydrogenase

3-hydroxybutyrate

Triple enzymatic system: Alcohol dehydrogenase-oxidoreductase–luciferase is the most sensitive to *quinones* and *organophosphorous pesticides (0.13–11 mg/L)*



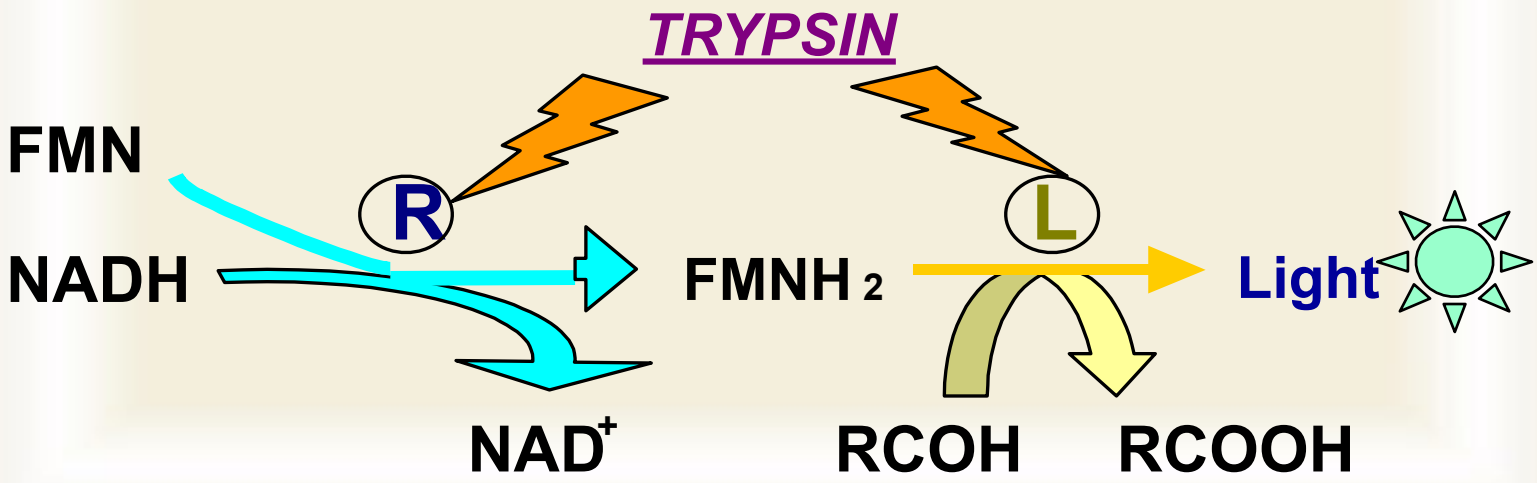
There was good correlation between **bioassays results** and hydrobiological data especially **sum biomass of blue-green algae** (*Microcystis aeruginosa, Aphanizomenon flos-aquae* and others)

Blooming in water ecosystems

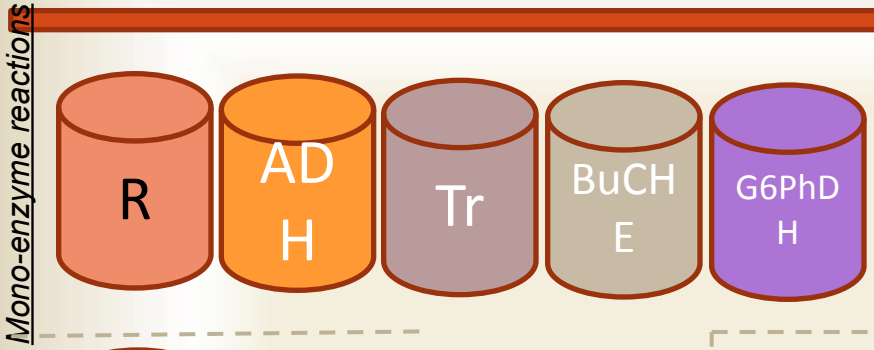
BEST™ Designer Bioassays

Triple enzymatic system

NADH:FMN-oxidoreductase and trypsin are the highly sensitive to lipotropic poison, a derivative of dithiocarbamine acid (0.03 mg/L) and may be used as indicator for **nerve gas agents (Sarin and Soman)**



Test-objects: 5 mono-, 1 bi-, 4 –triple enzyme systems



R – oxidoreductase
ADH – alcohol dehydrogenase
Tr – trypsin
BuCHE – butyrylcholinesterase
G6PhDH – glucose-6-phosphate dehydrogenase
Luc – luciferase
LDH - lactate dehydrogenase

Reference (control) soil samples

17 uncontaminated control samples of soil of different composition and characteristics based on sand and loan (light, medium and heavy) with different grain size and humus content.

Model pollutants

- **Metals**
  - copper chloride (II)
  - copper sulfate(II)
- **Pesticides**
  - «Decis»
  - Malathion
  - Diazinon
  - γ-isomer of hexachlorocyclohexane (HCCH)
- **Hydrocarbons**
  - Diesel fuel
- **Nano-materials**
  - titanium dioxide

The effect on the activity of enzyme systems was evaluated with IC_{20}, IC_{50} - the concentrations of the analyzed substances that inhibit the enzyme activity by 20 and 50%, respectively.

The enzymatic bioassay design for soil contaminations

√ Experimental model

SOIL

Grading

The humus content, %

Level of pollution

Model toxicants of different classes, given concentration

LIVING ORGANISM

Mono-enzyme reactions

butyrylcholinesterase

Two-enzyme reaction

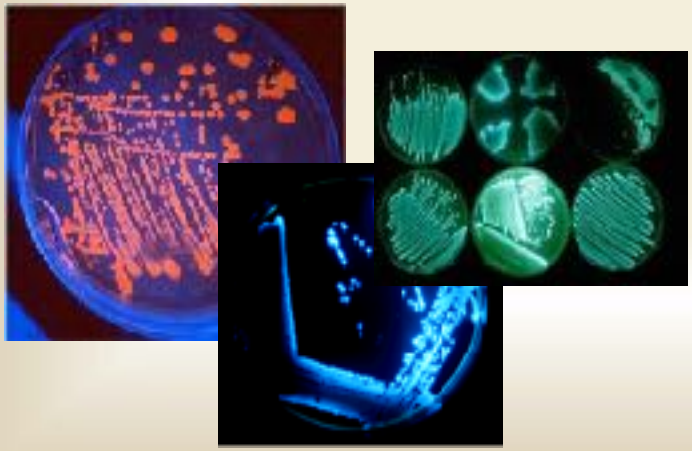
NADH: FMN-oxidoreductase + luciferase

Three-enzyme reaction

NADH: FMN-oxidoreductase + luciferase + alcohol dehydrogenase

The enzymatic toxicity tests

Map of snow pollution in Krasnoyarsk



- ***Environmental control***
  – natural ecosystems and industry wastewaters, soil and air quality
- ***Medical diagnostics***
  – endotoxicosis (blood plasma and serum, saliva),
  – sports medicine (athletic coaching control)
- ***Safety monitoring, control of food quality***
  – corn and bread infection by fungi
  – safety assessment of food additives
- ***Toxicology*** platinoids, pesticides
- ***Biotechnology***
  – safety monitoring of new materials (nanomaterials etc.)
- ***Scientific research***
- ***Education*** "Light as a language of life"

The bioluminescent laboratory “Enzymolum”

- **Manuals “Bioluminescent practical course” for high schools and universities (Master program “Biological Engineering”)**
- **Reagent “Enzymolum”**
- **Portable bioluminometer “LumiShort”**

Conclusions

Can living organisms in bioassays be replaced on enzyme?

- The new complex enzymatic biotests for environmental, healthy and other monitoring to screen the toxicity show many advantages
- Laboratory (portable bioluminometer, stable reagent, methods) is ready for using and market.

- We are looking for partners!!!

Laboratory of bioluminescent biotechnology

Go to Discovery together!!!