ENZYMATIC MARKER FOR TOXICITY OF CONTAMINATED SOILS

S. Fleschin, A. Bunaciu and Mihaela Scripcariu*

abstract: The treatment of toxic wastewater and remediation of soil is greatly facilitated by oxidizing the chemicals with selected activated sludge or mixed bacterial strains. Many different chemicals are destroyed or degraded to less hazardous products by altering the chemical structure of the molecule through reaction with the powerful hydroxyl radical. This simple to use oxidant power of microorganisms is easy to prepare, is inexpensive and is used at ambient temperatures and pressures..

Introduction

The concept of applications of enzymatic determination in wastewater treatment or soil remediation is reconsidered now in modern way. The treatability of any industrial wastewater, of course, depends upon its composition. A wastewater containing any of the compounds susceptible to enzymatic oxidation is a candidate for treatment [1,2].

Industries produce wastewaters which contain some toxic organics susceptible to oxidation. For example, the petroleum industry produces waste streams containing phenols, benzenes, toluens and xylenes. Plywood and adhesive manufactures frequently have phenol, formaldehyde and resorcinol wastes. The aerospace industry uses a combination of chemicals including phenol, formic acid and benzoil alcohol in stripping paint from aircraft [3,4]. Modern industries generating wastewater with enzymatic treatment possibilities are listed below, in Table 1.

Experimental

It well known that dehydrogenases are a group of intra celular enzymes that are involved in microbial oxidoreductase metabolism. This enzymes have been frequently used as an index of microbial activity in wastewater and soil samples [5,6].

The activity of such enzyme is linked to the respiratory and energy producing processes, in the cell, and basically depends on the metabolic state of microorganism.

* University of Bucharest, Department of Analytical Chemistry, 76229, 90-92, Sos. Panduri, Bucharest, Romania

Analele Universităţii din Bucureşti – Chimie, Anul XIII (serie nouă), vol. I-II, pag. 45-48

Determination of dehydrogenase activity in activated sludges or soil sample is usually based on the use of tetrazolium salts as artificial electron acceptors, which are reduced by microbial activity to red coloured formazans, which can be determined spectrophotometrically [6-8].

By choosing appropriate conditions the natural H^+ / e^- acceptors of the microorganisms of soil or sludge samples are replaced by the redox dye triphenyl tetrazolium chloride. Respiration of the microorganisms causes a reduction of TTC to triphenyl formazan TPF, which is red and insoluble in water. Concerning soil and sludge sample, TTC has been applied exclusively in the determination of general dehydrogenase activity.

Table 1

Civilian industries	Military industries
Aviation paint stripper wastewater	Air force, Navy Coast Guard, paint stripper waste water
Aircraft and engine cleaning waste water treatment	Engine cleaning wastewater
Electroplating circuit board wastewater	Electroplating circuit board wastewater
Plastic industry chemical process waste water	Rocket fuel waste water
Refinery phenol	Explosives waste water
Dye waste water	Remediation of contaminated soil
Textiles waste water	
Pesticide production and application	
Drugs and pharmaceuticals	
Wood treating	
Remediation of contaminated soils	

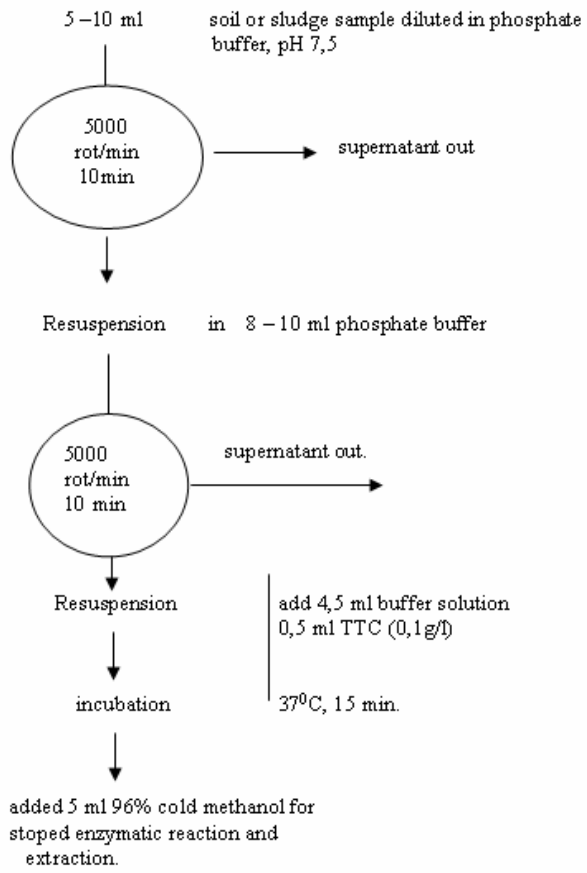
Table 2. Redox processes in soil and sludge sample.

Reaction	Redox potential *p*H = 7,0 ; 1 atm
$4H^+ + 4e^- + O_2 \longleftrightarrow 2H_2O$	E_0 = + 800 mv
$TTC + 2H^+ + 2e^- \longleftrightarrow TPF$	E_0 = + 490 mv

In this case, TTC replaces oxygen as final H^+/e^- acceptor in an aerobic mixed culture of bacteria from soil or waste water sludge. TTC is significantly reduced only by the aerobic cytocrome system.

Procedure for enzymatic determination

Enzymatic determination is shown schematically bellow:



Results and Discussion

Spectrophotometric procedure for calibration curves is very simple.

Standard solutions with 0; 5; 10; 20; 30; 40 μg TPF/ml were made in methanol. The UV-VIS spectra of TPF standard solution in methanol shows a principal absorption peak at 480 nm, which was the wavelength for the spectrophotometric readings of supernatant samples (Fig. 1).

Dehidrogenase activity was quantified by measuring the optical density of the supernatant against the blank at 480 nm.

The preliminary results was obtained with two sample of soil from a little garden 10 km from Brazi factory and soil from treatment station of waste water whose belong phenol factory.

In Fig. 2 are represented the variation of the absorbance at 482 nm with the amount of TPF added for this soil samples.

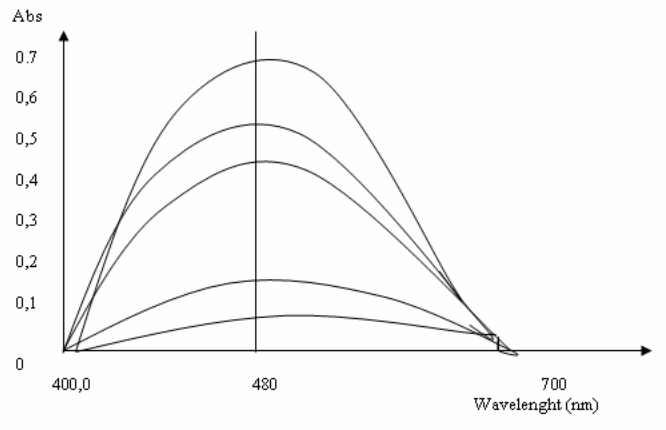


Fig. 1 *UV-VIS spectra of TPF in methanol.*

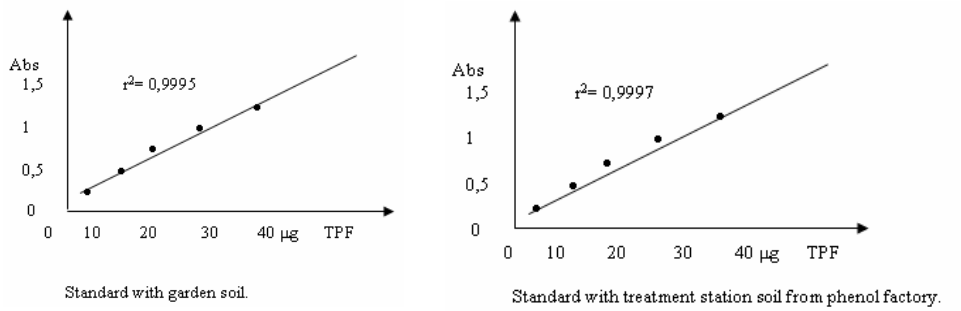


Fig. 2. *Variation of the absorbance at 482 nm with the amount of TPF.*

Conclusion

Small differences in absorbance were observed between the standard with garden soil and treatment soil (r = 0.9995 and 0.9997 respectively) which indicates a good extraction with methanol and presence of phenol in the proximity (10 km) of Brazi phenol factory.

REFERENCES

1. Hoffmann, D.J., Rattner, B.A. and Burtom, G.A. (1994) **Handbook of Ecotoxicology**, Boca Raton.
2. Benka-Coker, M.O. and Ekudayo, J.A. (1998) *Bioresource Technology* **66,** 241.
3. Torslov, J. (1993) *Ecotoxicology and Environmental Safety* **25**, 33.
4. Brohon, B. and Gourdon, R. (2000) *Soil Biol.Biochem* **3**, 853.
5. Camina, F., Trasar-Cepeda, C. and Gil-Sotres, F. (1998) *Soil.Biol.Biochem* **30**, 1005.
6. Alefp. Nannipieri, K. (1999) **Methods in Applied Soil Microbiology and Biochemistry**, Academic Press.
7. Tam, N.F.Y. (2001) *Environmental Pollution* **102**, 233.
8. Schimmel, S.C. and Morrison, G.E. (2001) *Environ. Toxicol.Chem*. **8**, 739.