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DEHYDROGENASES ACTIVITY IN SLUDGE SAMPLES OF SUCEAVA RIVER

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Abstract

To study the pollution level in water of Suceava river, the dehydrogenases activity of the sediments has been evaluated. Samples have been gathered from three sites, Brodina, Mihoveni and Tisauti. There had been determining the actual dehydrogenases activity and potential one respectively, and on the other side the activity of malat-, α-cetoglutarate-, succinate-, and isocitrate-dehydrogenases respectively, enzymes implied in Krebs cycle of oxidative degradation. Dehydrogenases are components of the enzyme systems of microorganisms, playing an important role in the energy production of organisms. Their role is to oxidize organic compounds through the transfer of two hydrogen atoms. Due to this dehydrogenase activity can be used as an indicator of biological redox systems and also as measure of microbial activity in soil.

Keywords: *Dehydrogenases; Sludge; Sediments; Water pollution.*

Introduction

Microbial communities act like factories in ecosystems, because they recycle the substances, degrade pollutants and are sources of carbon and energy [1]. Also their activity is closed by level of organic matter, sediments and micro-flora. Bacterial plankton has a role in organic matter mineralization and water self-purification. Micro flora is closed by pollutants level because reacts quickly when pollutants concentration change, so many authors evaluate water purity and pollution level function of number of microorganisms (including those saprophyte) [2]. Distribution of eco-physiological groups is characterized by significant differences due to temperature, quantities of organic matter, pH and water flow, because these parameters affect the activity of enzymes from the sampled organic slime.

Changes in microbial communities as a reaction to anthropic factors (wastewater discharge, for example) are followed usually by changes of three components of rivers: water

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flow, surface sediments and for those matter sediments. The structure of bacterial community is spatially and temporally characterized by strategic distributions **r/K** [3]. Malcom and Stanley consider sediments like a complex under action of different factors, like major mineral matrix, texture, quantity of organic carbon and proper location [4]. Cited authors point that slimes have three major components: detritus material (derived from soil erosion), biogenic material (derived for biological activity) and autogenic material (formed in situ); final character of the slime is closed by relative rates of these components.

Based on season and climatic conditions significant differences between sedimentary fractions could exist. Geo-morphological configuration of the basin in hills and planes regions determines the benthos structure and further the enzymatic activity. Also, considering that number of bacteria is higher in sediments comparing with water flow, on could conclude that significant processes of organic matter degradation occurs in slimes, closed by organic matter concentration, nutrients and oxygen saturation.

Quantitative variation of oxygen used for aquatic microbial oxidoreductases activity depends by oxygen quantity from aquatic plants photosynthesis, temperature, deepness and stratification of water, organic substances charge and reducing agents charge [5]; scientific literature points that oxygen demand is the basic element of correlation between organic matter quantity from water and activity of microorganisms, maximum values being registered in June, July and August while minimum values being registered during winter period, that means microorganisms activity is strongly coupled with temperature. Enzymatic profile of microbial population is characterized by activity of redox enzymes, especially by total dehydogenasis activity [6].

Normally, Suceava River has a good ecological status as far as concern pollution (stage 2) [7]. Specialized studies show that scarce level of metals proves a low pollution of the river, but nutrients from Tisauti section are in concordance with discharge of treated wastewaters from Suceava town mains [8, 9].

Anthropogenic activities could also influence the rate of microbial activity by increasing of metallic pollutants concentration, these being benefic for microorganisms or acting like inhibitor. Sometimes microbial population could use pollutants like electrons donors or acceptors in energetic metabolism, converting pollutants through enzymatic reactions into non-toxic substances [10].

The slime of the rivers is not only an alimentary material for microorganisms, but also a reservoir for a variety of contaminants, because aquatic slime adsorb chemical substances at high levels comparing to water column. From biochemical methods for monitoring microbiological activity, those closed to dehydrogenases activity are preferred because in methods closed to supervising microorganisms activity some difficulties appear, like estimation of number of viable cells [11-13].

Dehydrogenases activity point the oxidative capacity of the slime because these enzymes are present in all live cells and act in tricarboxylic acids cycle. These enzymes are located strictly intracellular, being active only in viable cells.

It has to consider the fact that a normal dehydrogenases activity or a reduced one in slimes polluted with metals, did not signalize a low level of pollution, because microbial metabolism could be adapted to a long time exposure situation [14]; other authors consider that enzymatic activity inhibition is closed to a slime complexation and not to a tolerance to metals for microbial communities [15-18].

Material and methods

To determine dehydrogenases activity from the samples of sediments the colorimetric method based on Triphenyl-tetrazolium chloride (TTC) was used. The principle of method consists in reduction of uncolored TTC to red colored formazan under action of hydrogen transferred from dehydrogenases, extraction of it with organic solvents (ethanol, methanol and acetone) and spectrophotometric detection (485nm wave length). So color intensity is proportional with dehydrogenases activity.

Initially, trace of the calibration curve was obtained and then the regression equation was calculated. That curve allowed finding proper quantities of formazan for analyzed samples.

For every sample there were effectuated three parallel determinations, results were statistically analyzed and confidence intervals calculated.

All the samples were processed at the Biochemistry and Molecular Biology Laboratory from the Al.I. Cuza University, Iasi.

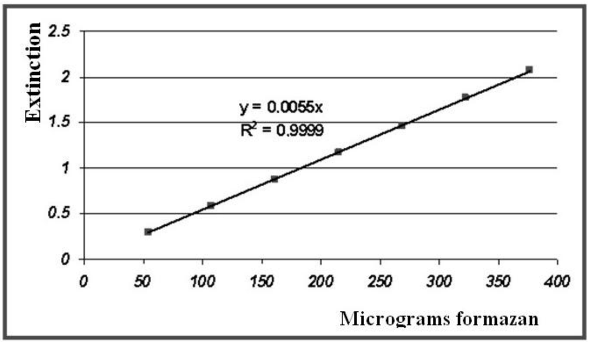

Fig. 1. Etalon curve for formazan quantity dosage

Results and discussions

Physical characteristics of the sediments have a strong influence against dehydrogenases activity by changes in aeration status of superior layer. Some data from literature show that dehydrogenases activity is inverse correlated with air percolation to slime surface, diffusion rate and redox potential. Hence dehydrogenases activity increases with aerobiosis.

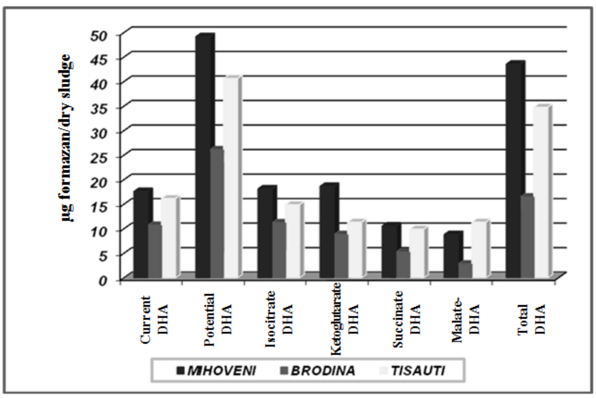

Fig. 2. Dehydrogenases activity corresponding to sediments from Suceava River in 2008 - sites Mihoveni, Brodina and Tisauti

Dehydrogenases have founded in all test portions in 2008 and they reflected accuracy the intensity of respiration at the sediments level of Suceava River in sites Mihoveni, Brodina and Tisăuţi. Analysis of data emphase a significant difference between actual dehydrogenase and potential dehydrogenase, what could be explained by an intense respiratory process in sediments coupled with a supplementary nutrient material entrance [19, 20]. A diminution of actual dehydrogenases activity between sites Mihoveni and Brodina could be explained by a possible pollution and a perturbation of sediments whose organic matter decreased. Another explanation of the phenomenon could be sand from Brodina, Suha site like that is rare in organic matter and do not allow microbial proliferation biotope, which differs from other surface waters with inorganic chemical loading [21-27].

Values recorded for Brodina site of 10.753μg formazan/g dry sludge for actual dehydrogenase and 26.154μg formazan/g dry sludge for potential dehydrogenase respectively, could signalize a microbial biotope adapted better to pollution comparing to Mihoveni, because dehydrogenase activity of Gram-posite bacteria is higher than dehydrogenase activity of Gram-negative bacteria, even previous reports contested this affirmation.

Observed variations could appear due to bacterial physiology, including cellular membrane or dehydrogenases system, because for different microorganisms there were signalized different dehydrogenases systems. So, for Mihoveni site actual dehydrogenases activity was 17.641μg formazan/g dry sludge, what represents a third of potential dehydrogenases activity (49.234μg formazan/g dry sludge). This could be explained by high level of pollution of water in that location.

Instead of it, in Tisauti site dehydrogenases activity was 16.203μg formazan/g dry sludge for actual activity opposite to 40.685μg formazan/g dry sludge for potential activity one, both of them being higher comparing with Brodina site, but lowest comparing with Mihoveni.

The values for total dehydrogenases activity concords with those obtained for separate determination of oxydoreductases which are catalysts for the principal stages of Krebs cycle, malat-α-cetoglutarate-, succinate-, and isocitate-dehydrogenases respectively. An equilibrated tricarboxylic acids cycle could be noted for all three sites. As an enzyme which reflects respiratory rate, isocitrate-dehydrogenase registered high amplitude for all three sites 18.191μg formazan/g dry sludge (Mihoveni), 11.265μg formazan/g of dry sludge (Brodina) and 14.950μg formazan/g of dry sludge respectively (Tisauti).

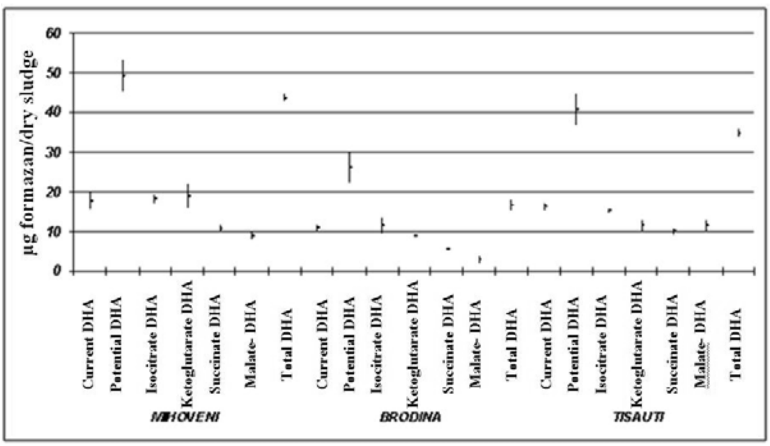

Fig. 3. Confidence intervals for dehydrogenases activity corresponding for 2008 in sediments of Suceava River – sites Mihoveni, Brodina and Tisauti

From the graphic chart one could observe that limits of confidence for actual dehydrogenase activity, potential dehydrogenase activity, but also for isocitrate-, ketoglutarate, succinate- and malate-dehydrogenase are very restricted for all considered sites. Highest variability intervals are registered for potential dehydrogenase activity, being of 45.231 – 53.272μg formazan/g dry sludge for samples from Mihoveni area, of 22.152 – 30.15μg formazan/g dry sludge for Brodina and of 36.681 – 44.592μg formazan/g dry sludge for Tisauti site respectively.

Conclusions

Dehydrogenases activity is a good marker of microbial activity and could signalize pollution of waters without measuring every parameter. Some cautions have to be taken, because microbial populations could adapt their metabolism to a high concentration of metals. Correct interpretation of data has to allow for variation caused by temperature, sediments morphology, seasoning and other specific parameters.

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