

MICROBIAL CULTURE ADAPTATION TO BIODEGRADATION OF DIMETHYLTEREPHTHALATE

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Summary

45 microbial cultures have been isolated from a chemically polluted region, using dimethylterephthalate (DMT) as a sole carbon and energy source. They have been subjected to adaptive selection in order to increase their biodegradative potential. The gradual increase of the xenobiotic substrate concentration from 2.5 to 25 mM induced adaptive changes in the tested strains. 20% of the studied strains showed a high biodegradative activity towards DMT and the best adaptation was expressed by the soil microorganisms.

Introduction

The phthalic derivatives are one of the most widely spread environmental pollutants. Their increasing utilization in some industries (especially in chemical and textile manufacture) places them in the group of the problem-arising xenobiotics, together with the chloroorganic compounds and pesticides [4]. The low solubility and bioaccumulation ability determine their presence in soil and water, as well as their accumulation along the trophic chains.

The role of the microorganisms in the

biological fate of this type of xenobiotics has not been entirely clarified yet and their biodegradation is a subject of investigation in many laboratories [2, 3, 9, 17]. This is also a current problem for our country, where it mainly concerns the pollution with DMT, an aromatic di-carbon derivatives group ester and major monomer in the production of the polyester fibre by trade name "Yambolen".

The aim of the present work was to isolate and adapt microbial cultures, catabolizing DMT as a sole carbon and energy source.

Materials and Methods

Microorganisms. Soil and water samples were taken from 3 places in the region of the waste water treatment plant of "D. Dimov" textile factory: strongly polluted soil, biobasin and equalizer. The samples were treated according to the conventional microbiological methods for obtaining of pure cultures using dilutions and inoculation on solid medium with methylbenzoate as a carbon source. The culture was a part of the internal collection of the

NBIMCC, Bulgaria. It was stored on a slant agar at 4-6°C and passaged each month.

Chemicals, reagents and media. DMT and methylbenzoate were kindly supplied by the chemical laboratory of the Textile Manufacture (purity 99.9%). The system for evaluation of the optical density at λ_{450} consisted of ethanol: butanol: chloroform =

10:10:1. A mineral medium with composition [1] (g/l): K_2HPO_4 - 1.82; NH_4NO_3 - 1.0; $MgSO_4 \cdot 7H_2O$ - 0.2; $CaCl_2 \cdot H_2O$ - 0.02; $FeSO_4 \cdot 7H_2O$ - 0.0006; $NaMoO_4 \cdot 2H_2O$ - 0.06 and $MnSO_4$ - 0.06 in distilled water, pH 7.15 was used in the experiments. The carbon sources were added to the mineral mixture under sterile conditions either directly for the methylbenzoate, or as concentrated ethanol solution for DMT.

Cultivation and measurements. The microbial culture adaptation, aiming at a selection of active biodegradants of DMT, was performed in 2 steps. The isolation and the first adaptation process were carried out on solid mineral medium with increasing concentrations of methylbenzoate as a sole carbon and energy source. The adaptation con-

tinued in liquid medium with carbon source DMT in batch culture. DMT concentration was increased gradually. The determination of dry weight, optical density or number of cells/ml was used as a criterion for quantitative evaluation of microbial growth.

The tested microbial strains were cultivated on a rotary-shaker "Gallenkamp" with 200 rpm at 26-28°C. 50 ml mineral medium were inoculated with cell suspension (10^9 cells/ml). The biomass accumulated during the biodegradative process was evaluated by the dry weight measurement. An aliquote (10 ml) was filtered through "Millipore" DA-0.65 filter and dried at 105°C to constant weight. Meanwhile the OD at λ_{450} was determined using a dissolving system, that did not damage the cells and allowed total solubilization of the aromatic ester [10].

Results and Discussion

Fourty five microbial cultures were isolated from permanently treated with DMT biotopes - 18 from soil near to the waste water treatment facilities, 15 - from the biobasin and 12 from the equalizer. The methylbenzoate, a product currently connected with the production technology and close in chemical structure to the DMT, at concentration 0.73 mM was used as a sole carbon and energy source in terms to induce adaptative changes in the microorganisms. This concentration was increased 2, 5 and 10 times (1.47, 3.67

and 7.34 mM, respectively). The studied microbial cultures showed high adaptation abilities to catabolize this aromatic xenobiotic, and growth was registered in 78% of the total number of the microorganisms (Fig.1a) even at the highest tested concentration (7.34 mM). The solid medium on which this initial adaptation period had been performed, allowed "gentle" conditions for development of the microbial cells due to lack of direct contact with the aromatic ester. In this way, a set of microorganisms with valuable for the prac-

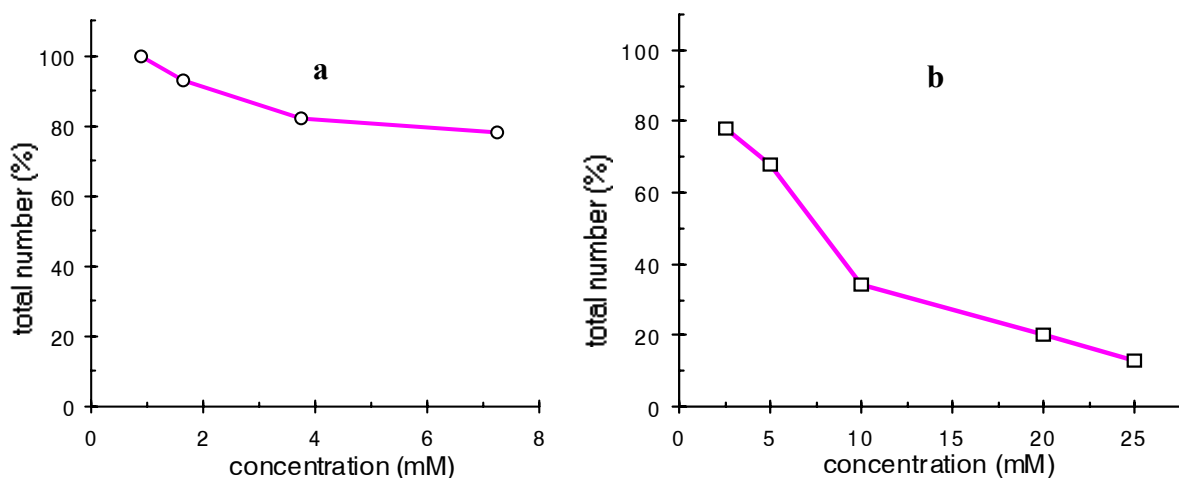


Fig.1. Influence of growth substrate concentration on the total number of microbial cultures during the adaptation process; **a** - methylbenzoate in solid medium, **b** - DMT in liquid medium.

tice metabolic properties was selected. Further, active biodegradants of DMT were chosen from this group.

The next stage in the adaptation process of the studied microorganisms was batch cultivation in liquid medium with increasing concentrations of DMT as a sole carbon and energy source. The aromatic substrate was decomposed by the tested cultures at 2.58 mM and 5.15 mM. 10.3 mM terephthalic ester in the medium inhibited the development of 67.7% of the total number of cultures and can be evaluated as a "threshold" concentration for selection of microorganisms with considerable biodegradative potential towards this aromatic substrate (Fig.1b). The relatively low DMT toxicity is a possible reason for the high concentration values at which an inhibitory effect on the microbial growth was performed. Like in other cases of microbial adaptation for xenobiotic decomposition (chlorine-containing aromatic compounds [7, 12, 15, 18], biphenyls [14], benzoates [6], etc.) an obligatory reorganization of the catabolic apparatus also takes place in this case. Such an ability gives the microorganisms a great possibility for adaptation and decomposition of compounds, foreign to the cell but available in the environment due to anthropogenic reasons.

The general adaptation response scheme of the tested cultures in liquid medium is presented in Fig.2. The initial adaptation period was considerably prolonged from 7 to 14 days (for some moulds). Obviously, series of changes on subcellular, cellular and population level leading to development of a clone with the best degradative activity towards the tested xenobiotic, took place during this period of time.

In the next passage of the already adapted cell suspension, the lag-phase was shortened, regardless of the increased DMT concentration. The practical value of the adaptive changes in the biotechnologically perspective strains consists especially in the shortened decomposition period of the non-typical substrate. A 10-15 fold increase of the

detoxication processes rate after adaptive changes, has been observed by other authors [8, 11]. This phenomenon is explained with the increased intensity of metabolic processes in the presence of inductor in the medium. It is well known that the substrate induction is a basic regulatory mechanism in the biosynthesis of the catabolic enzymes in the biodegradation of xenobiotics [5].

The higher DMT concentration in the medium (20.65 and 25.75mM) were utilized by 20% and 13% of the total number of the tested cultures respectively. These values are in agreement with the data of Slyzen et al. [16] concerning the biodegradation of DMT in concentration 10 g/l and those of Naumova for the terephthalic acid - up to 7.75 g/l [13]. An increase in the concentration of DMT over 25mM has not been tested, because such high values are as a rule not existent in the industrial waters even after accidental contaminations.

The number of the microbial cultures at the different adaptation levels, depending on the isolation biotope is presented in Fig.3. The cultures isolated from the soil clearly represented better abilities for adaptation. They showed remarkable adaptive capabilities and great flexibility of their metabolic apparatus depending on the various conditions appearing in their native environment (availability or lack of nutrients, influence of unfavorable abiotic factors, etc.). However, in our opinion it is possible that the relative inertness in the adaptive response of the cultures, isolated from the biobasin and the equalizer is due to the permanent control of some parameters (pH, concentration of biogenic elements, etc.) in the waste treatment pool and their contribution in providing better conditions for development at stress conditions.

As a conclusion it can be summarized that the isolated microbial cultures are a perspective object and their biotechnological application in waste water treatment is possible after a better characterization.

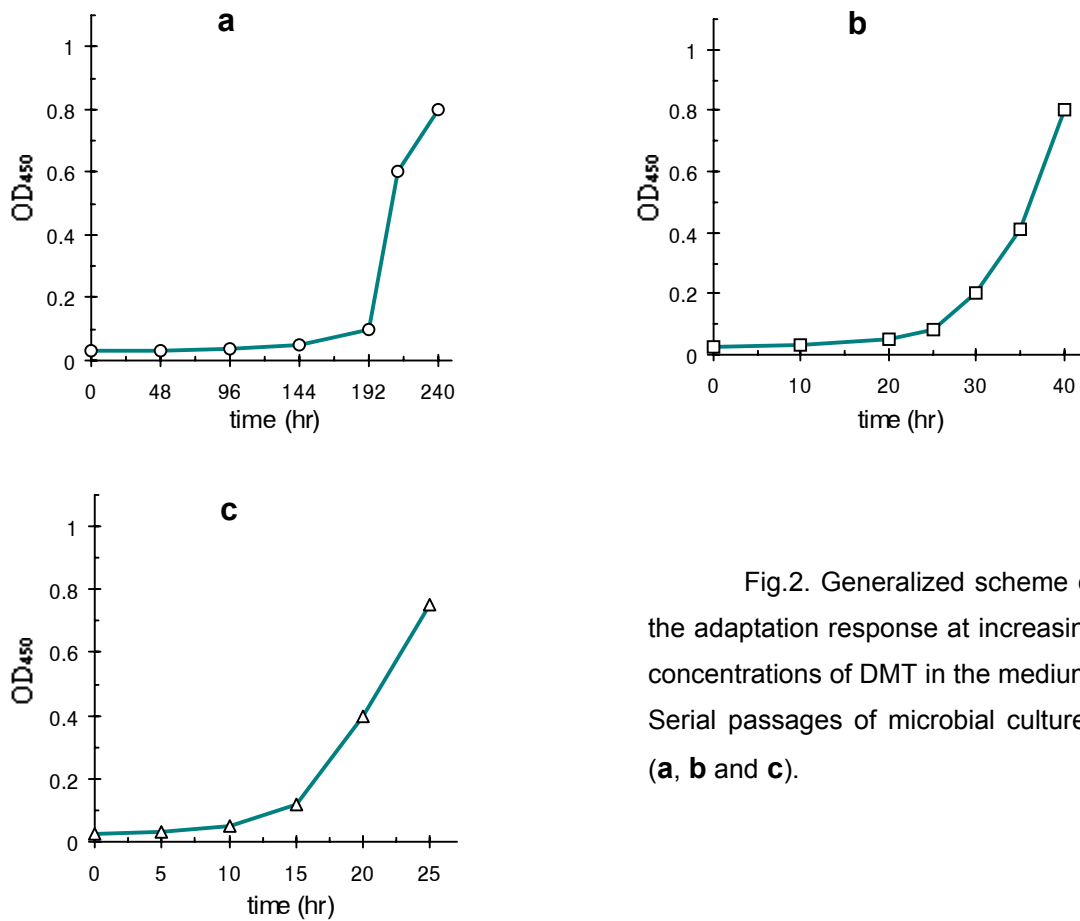


Fig.2. Generalized scheme of the adaptation response at increasing concentrations of DMT in the medium. Serial passages of microbial cultures (a, b and c).

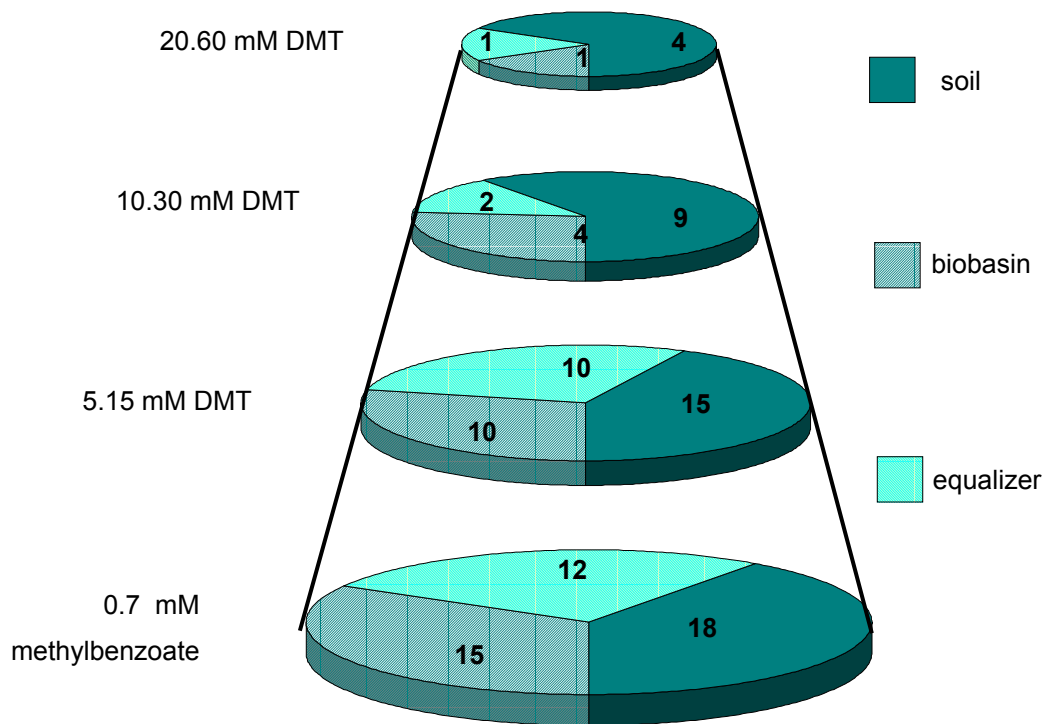


Fig.3. Number of the microbial cultures on different adaptation levels depending on the isolation biotope.

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