

DEGRADATION OF DIMETHYLTEREPHTHALATE BY NATURALLY FORMED MICROBIAL ASSOCIATIONS 169AC AND 189AC

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Summary

Mixed microbial cultures, naturally combined in two-member associations (169AC and 189AC), actively degraded the aromatic ester dimethylterephthalate as a single source of carbon and energy. The biodegrading activity of the associations and also of the pure culture combination was tested under polysubstrate conditions. When complementary substrates like methanol, xylol and ethylene glycol were added, the consisting of a bacterial and an actinomyces strains association 189, exhibited a higher activity. A biomass of this association was brought to freeze-drying and the lyophilizate saved biodegrading activity towards the studied substrate.

Introduction

The orthophthalic acid diesters are an important group of industrial chemicals that belong to the priority pollutants of the environment [2]. The meta- and the para-isomers of the phthalic acids and their esters are produced in limited quantities, but they also have a considerable industrial application. The terephthalic acid dimethyl ester (DMT) is used as a key crude material for the production of various polyester fibres like trevira (in Germany), terilen (in England), Dacron (in USA). In our country, jambolen is produced based on this ester.

The DMT is significantly less investigated than the orthophthalic derivatives. Despite the insufficiency of data for its toxicity, bioaccumulation and biodegradation, its

mutagenicity is proved [14]. In scientific literature a few authors [1, 6, 7, 9] report about the biodegradation of DMT, but the attempts to use active phthalatedegradings for the assimilation of DMT give no positive results [3]. This was the reason why the possibilities for its biodegradation and the purification of waste industrial waters from this substrate were of interest for us.

The aim of the present work was, by the use of adapted microbial cultures, as well as their lyophilizates, to investigate the biodegradation of the aromatic ester DMT under polysubstrate conditions, directly correlated to the concrete physicochemical situation in the biobasin of the textile industry purifying equipment.

Materials and Methods

Microorganisms. Two mixed microbial cultures 169AC - consisting of two bacterial strains, and 189AC - consisting of a bacterial and an actinomyces strains, were the object of the present work. They were obtained as a result of adaptive selection of naturally formed associations, actively degrading DMT. They were isolated from polluted soil in the region of the Textile Factory - Jambol, and additionally adapted to higher concentrations (50 - 5000 mg/ml) of aromatic substrate [12].

Media and cultivating conditions. The mineral medium, used in the experiment, contained in g/l: K_2HPO_4 - 8.25; KH_2PO_4 - 1.82; NH_4NO_3 - 1.0; $MgSO_4 \cdot 7H_2O$ - 0.2; $CaCl_2 \cdot 2H_2O$ - 0.02; $FeSO_4 \cdot 7H_2O$ - 0.0006; $NaMoO_4 \cdot 2H_2O$ - 0.06; $MnSO_4$ - 0.06. To the basic medium, as a source of carbon and energy, DMT was added as a concentrated ethanol solution (50 mg/ml), while the complementary sources of carbon (methanol, para-xylol and ethylene glycol) were directly added. The cultivation was performed periodically on a shaker "Galencamp" (200r/min) at 28°C. The aromatic substrate biodegradation by associations 169AC and 189AC was presented

in former publications of ours [12, 13].

Quantitative determination of DMT.

The determination of the residual quantity of DMT was realised by the use of a modified by us colorimetric method of Goddu for the confirmation of traces of complex esters [8]. After three-fold extraction and drying, the residual quantity of ester was processed in accordance with the method and the colour reaction was registered at 540 nm on SPEKOL 11 [12].

Biomass determination. The determination of the accumulated biomass quantity was done by the use of the weight method through drying at 105°C until constant dry weight.

The 189AC biomass freeze-drying was performed in lyophilizer SMH - 15 (USIFROID) at the following regime: freezing down to minus 32°C with a speed of 0.6°C.min⁻¹ and sublimation under pressure 17 Pa; final drying at 22°C over 6 hours under a pressure of 2 Pa.

The presented experimental results are average of three-fold repetition. When the analysis data were statistically processed, the level of reliability was 95%.

Results and Discussion

Having in mind the complex character of the textile production waste waters, we moulded out a polysubstrate system with a proportion of the pollutants deduced from the data for volley pollution in the station. DMT, ethylene glycol, methanol and para-xylol were present in the medium as carbon components, in a ratio 1:0.2:0.2:0.01. The growth of 189AC association and the main substrate degradation are exhibited on Fig. 1. A slow down of the biodegradation was observed on the average of about 10 hours and this could be explained with the complicated conditions when combining the positive and inhibitory influence of the different substrates on the microbial activity. However, a block out of the process was not registered and

this fact was considerably essential from a practical point of view.

When the biodegrading activity of the two associations (169AC and 189AC) was compared, an advantage to the mixed culture 189AC could be given. The actinomycetes strain in this co-culture had a leading role in the DMT degradation. We combined this strain with the two-member association 169AC under laboratory conditions (Fig. 2). The metabolic activity of the resulting mixed culture was investigated when combining the main substrate DMT with the three complementary ones - methanol, para-xylol and ethylene glycol, and was compared with the one of mixed culture 169.

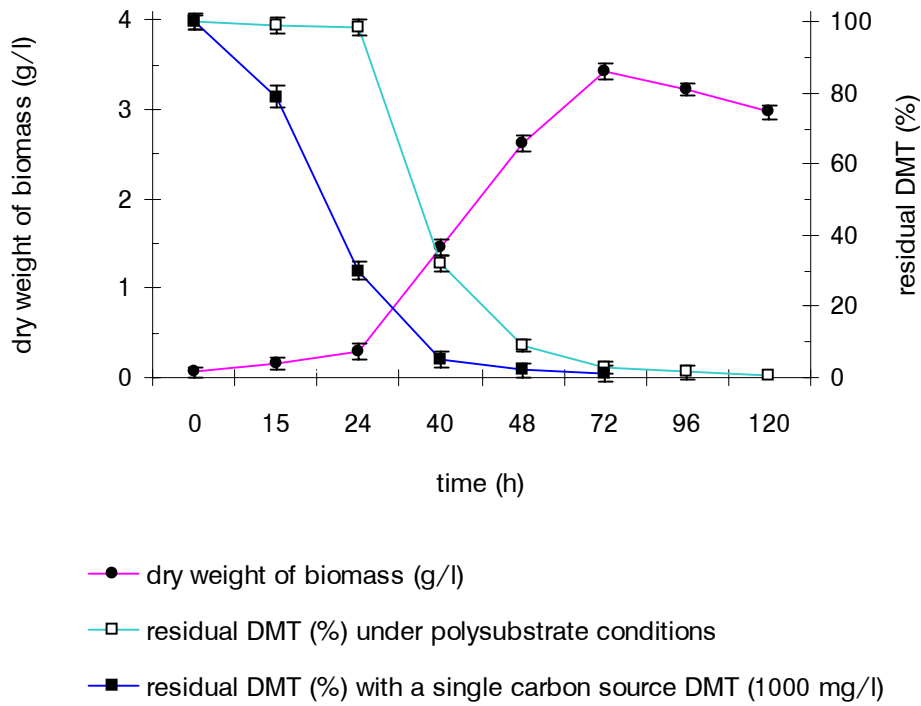


Fig.1. DMT biodegradation by association 189 under polysubstrate conditions.

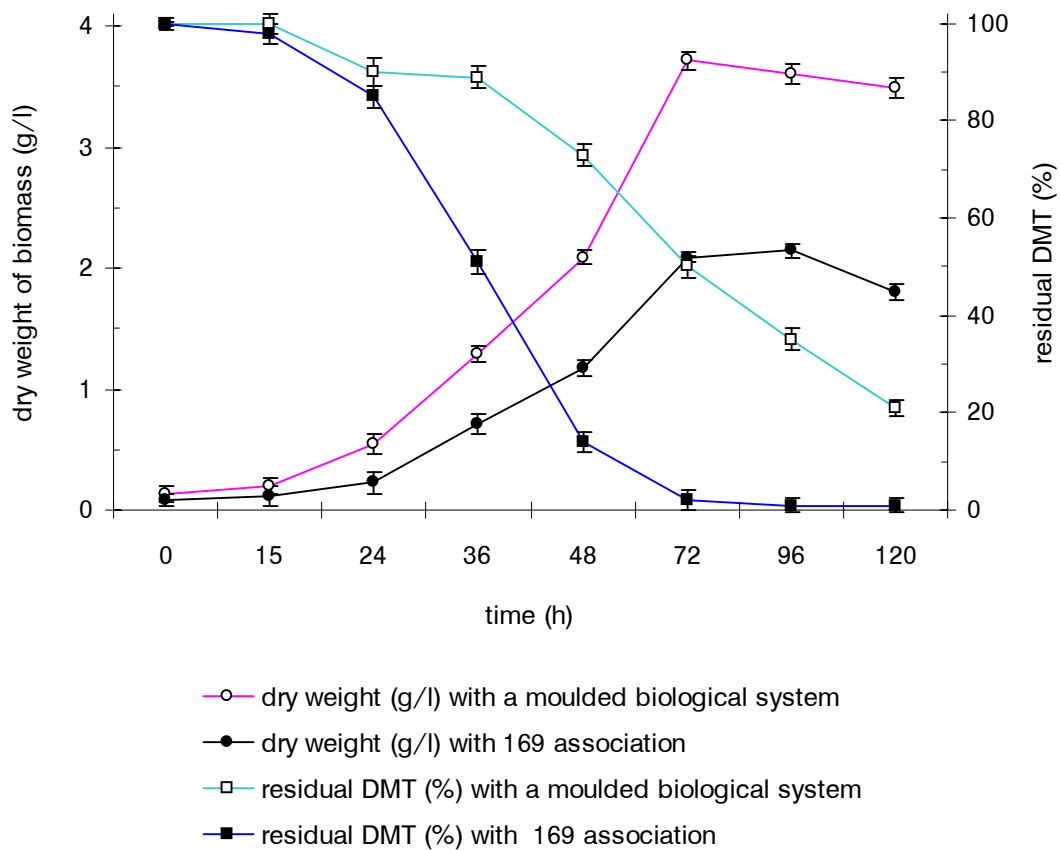


Fig.2. DMT biodegradation under polysubstrate conditions, by 169 association, inoculated with actinomyces strain 189-AM.

Fig. 2 definitely shows that under polysubstrate conditions, association 169AC performs the biodegrading process in a more difficult way. This was a result of the variety in the type of the growth substrates and also of the high organic loading. About 50% of the main substrate were eliminated until 72nd hour. Under the same conditions, with the newly in an artificial way formed association, 98% of the DMT were catabolised. This was indicative of the actinomycetes leading part in the process of oxidation. These results brought to the idea about the introduction of the actinomycetes member, or for better of the whole 189AC association as a stabile natural formation, to the activated sludge of the purifying biobasin. Kurane [4, 5] reported for a similar inoculation in the activated sludge of an actinomycetes strain from genus *Nocardia*. The activated sludge, enriched with *Nocardia erythropolis*, degraded the di-2-ethylhexyl-phthalate 100% within three days, while without inoculation, the phthalic ester was not completely eliminated even after a one month period of adaptation.

The next experiments of ours were connected with the technological side of the investigated problem. The application of lyophilized biopreparations in cases of average pollution of world-wide significance is widely used. The 189AC association, being the most active and stable in the biodegrading process, was again the object of the investigations. It was put to lyophilization and the microbial preparation DMT degrading capability was tested in two variants, in accordance with the quantity of the added lyophilizate (0.5 and 0.05 g/l) - Fig. 3. The biodegradation was performed faster in the first case.

These results of ours were comparable to laboratory investigated lyophilized microbial preparations for nitrophenolic degradation [10, 11]. The added quantity of dry biomass in the quoted paper was of the order of 0.35 g/l.

The higher concentration of lyophilizate, 0.5 g/l, in our experiment, was more effective for the investigated substrate biodegradation. It was obvious that this was the proper concentration for the biotechnological application of the lyophilized culture. It was significant in this case, that the enzyme systems in the lyophilizate preserved their activity, which allowed the introduction of 189AC association when composing and producing a preparation, designed for application in the field of environmental biotechnology.

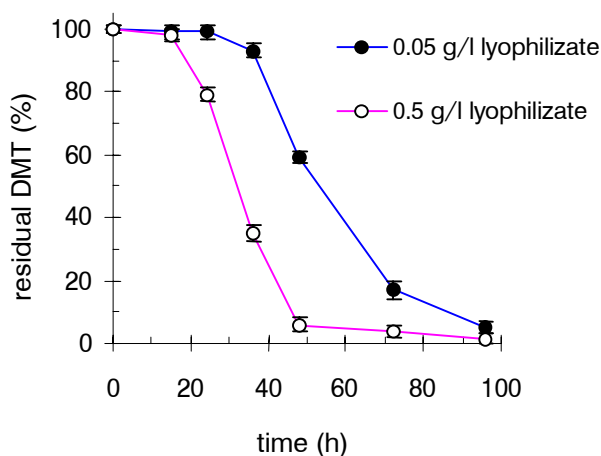


Fig. 3. DMT biodegradation by the use of lyophilized culture 189 AC.

As a conclusion, it can be generalised that 189AC association is stable and highly specialised in the degradation of the investigated aromatic substrate. The polysubstrate conditions do not bring to the loss of its activity and its metabolic potential can be used for intensification of the purifying process. Lyophilization seems to be a suitable method for its preservation and application for biotechnological treatment of textile industry wastewater.

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