Scientific article
UDC 579.63
DOI: 10.52957/2782-1900-2024-5-4-121-127

ASSESSMENT OF AROMATIC NITRO COMPOUNDS AND PHENOL BIODEGRADATION BY MICRO-MYCETES IN INDUSTRIAL EFFLUENTS

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Keywords:
biodegradation, aromatic nitro compounds, phenols, Trichoderma fungi, wastewater, utilisation, culturing

Abstract. *The paper analyses the experimental results of microbiological utilisation of mononitrotoluene and phenol at concentrations of 20, 50 and 70 mg/l by strains of Trichoderma fungi. According to the research, all the strains under study are able to grow in the presence of mononitrotoluene as the only source of nitrogen nutrition and phenol as the only source of carbon and energy. However, the strains have different sensitivity to changes in their concentration in the medium. Indeed, the strains under study are able to degrade mononitrotoluene and phenol in liquid medium. The maximum efficiency of mononitrotoluene biodegradation was 66% using Trichoderma koningii strain 'TSL-06', and that of phenol was 95% using Trichoderma harzianum strain 'M99/5'. Based on the results, we selected perspective strains for biodegradation of mononitrotoluene and phenol by surface and deep cultivation methods, as well as for the development of a biopreparation based on fungi of the genus Trichoderma in an immersion biotechnological system.*

For citation:
Bondar P.N. Assessment of aromatic nitro compounds and phenol biodegradation by micro-mycetes in industrial effluents // *From Chemistry Towards Technology Step-by-Step*. 2024. Vol. 5, Issue 4. P. 121-127. URL: https://chemintech.ru/en/nauka/issue/5563/view

Introduction

Nowadays, aromatic compounds dominate the chemical industry. The production volume of these compounds is up to several thousand tonnes per year [1].

Aromatic mononitro compounds are widely used in various branches of the chemical industry, but mainly in the production of amines. High reactivity of aromatic nitro compounds and amines provides their application for synthesis of synthetic dyes, production of polyurethanes, vulcanisation accelerators, antioxidants for rubber products, plant protection chemicals, pharmaceuticals, etc. Some aromatic nitro compounds are used as perfuming agents [2].

Wastewater containing, among others, mononitrotoluenes is treated, as a rule, by two methods: chemical and adsorption ones. The chemical method includes reduction of the nitro group by iron chips in an electrolyte medium and subsequent neutralisation of the acid with lime. Biological treatment is required to degrade the amines formed. Adsorption method by activated carbon allows ones to treat wastewater containing mononitrotoluene up to 500 mg/l

to its concentration of 30 mg/l. The adsorption process provides neutralisation of the acids with lime and desorption of the waste coal. The concentration of MNT in wastewater resulting in fish death in water reservoirs is 15-20 mg/l [1, 2]. In this regard, the degradation of nitroaromatic compounds is an urgent problem.

Phenol and its derivatives are actively used in almost all areas of industry: in the production of varnishes and paints, synthetic resins, plasticisers, surfactants and tannins, pesticides, stabilisers and antiseptics. Due to the intensive use of phenols, phenolic compounds are constantly present in the wastewater of many chemical enterprises, as well as coke, petrochemical, pulp, and wood processing industries [4, 5].

World production of phenolic compounds is about 50,000 tonnes/year. Phenol is classified as a toxic compound by character of action. The maximum permissible concentration (MPC) of phenol in water is 0.001 mg/l [6].

Nowadays, the most successful pollution control strategy is to utilise the ability of living organisms to assimilate and degrade xenobiotics [7].

The method of biological degradation using highly effective strains of microorganisms-destructors is recognised as a promising way of utilisation for such aromatic compounds as mononitrotoluene and phenol in wastewater. The advantage of using biological methods of destruction is based on the fact that microorganisms neutralise toxic substances without adversely affecting the ecosystem and without causing the creation of new polluting agents in the environment.

Most of the microorganisms of aromatic compound degraders described in the literature belong to various bacterial strains. However, the wide use of fungi of the genus *Trichoderma* in both activated sludge and biofilm, as well as their ability to utilise a wide range of carbon substrates, processability, relatively high growth rate and low toxicity to plants and animals, suggest the possibility of using these microorganisms for biodegradation of aromatic compounds [8-12]. According to many studies, Trichoderma genus fungi can be very resistant to toxic industrial environmental contaminants. In addition, they are also able to increase the efficiency of biodegradation of xenobiotics together with other destructor strains as a consortium [13-15].

Indeed, I. P. Solyanikova and co-authors [16] showed the ability of strains-destructors of various aromatic compounds to utilise nitrotoluene at concentrations up to 70 mg/l. We assumed this maximum concentration in the present study as well.

The purpose of this study is to investigate the effect of mononitrotoluene and phenol in different concentrations on the growth of *Trichoderma* fungi and select active strains for biological destruction of these toxicants during surface and deep cultivation.

Experimental part

We selected 5 monospore *Trichoderma* fungal strains for the research. These fungi were isolated from soils of different forest zones of Central Siberia and the Tyva Republic, Russia possessing stable cultural and morphological features and showing extracellular phenoloxidase activity: *Trichoderma asperellum* 'Mg-6', *Trichoderma asperellum* 'TH-11', *Trichoderma harzianum* 'M99/5', *Trichoderma koningii* 'TSG', *Trichoderma koningii* 'TSL-06'.

We used wastewater containing mononitrotoluene and phenol at concentrations of 20, 50 and 70 mg/l as study subjects.

To study the effect of mononitrotoluene and phenol, we used Chapek's nutrient medium of the following composition, (g/l): Glucose - 30.0; $NaNO_3$ - 2.0; $MgSO_4$ - 0.5; KCl - 0.5; K_2HPO_4 - 1.0; $FeSO_4$ - 0.01; agar - 20.0, containing mononitrotoluene at concentrations of 20, 50 and 70 mg/l instead of nitrogen-containing compound, or containing phenol at concentrations of 20, 50 and 70 mg/l instead of glucose. We autoclaved the medium at 0.5 atm for 30 minutes and cultivated the cultures in the thermostat at 25-27°C. We used the culture grown on Chapek's medium without addition of mononitrotoluene and phenol as a check.

We determined the concentration of mononitrotoluene in the medium before and after cultivation by chromatographic method using a liquid chromatograph 'Milichrom-2' [1].

We measured the concentration of phenol before and after cultivation using the photometric method [17]. The method is based on the formation of an orange-yellow complex of phenol with para-nitroaniline in alkaline medium. We transferred an aliquot of the analysed wastewater in a volume not exceeding 5 ml into a 25 ml flask. Then we added 1 ml of diazotised para-nitroaniline solution and added absorbent solution (sodium carbonate, 8 g/l solution) to the mark. We prepared diazotised para-nitroaniline as follows: 0.01 g of para-nitroaniline was dissolved in a mixture of 10 ml of distilled water and 2.5 ml of hydrochloric acid. We added 2.5 ml of sodium nitrate solution to the resulting solution and after a few minutes we diluted the solution with water to 50 ml; prepared the solution on the test day; determined the optical density at a wavelength of $\lambda = 440$ nm in a cuvette with a working length of $\lambda = 20$ mm, relative to the blank sample; performed all studies in three iterations.

Main body

Trichoderma fungi are highly species- and strain-specific. Therefore, to develop the basis for their use for biodegradation of aromatic compounds, it is necessary to screen them for sensitivity to changes in the content of toxic components in the medium [18].

We used surface culturing to initially assess the growth potential of fungi of the genus *Trichoderma* in the presence of different concentrations of mononitrotoluene and phenol. The surface method involves the culture growing on the surface of a solid moistened nutrient medium in the form of a mycelial film. It absorbs the ingredients of the nutrient medium by substrate mycelium and forms reproductive organs by air mycelium. This method of cultivation provides a complete cycle of fungal development, but is a slower process due to the intrahyphalic transport of nutrients from the substrate mycelium to the growing terminal cells of the aerial mycelium [19]. We applied the spores of the fungus by a loop injection into the centre of a Petri dish in medium and took the results on the 7th day.

Studies on the effect of mononitrotoluene as the only source of nitrogen nutrition on the growth of *Trichoderma* strains under surface cultivation conditions showed that they have different sensitivity to changes in its concentration in the medium. Therefore, the greatest productivity was observed for *Trichoderma asperellum* 'M99/5' and *Trichoderma koningii* 'TSG' strains at 20 mg/l and 50 mg/l concentrations of MNT in the medium, and at 70 mg/l - for *Trichoderma koningii* 'TSG'.

Similar results were obtained in the case of phenol influence as the only source of carbon and energy. The highest productivity was observed in *Trichoderma asperellum* 'M99/5', *Trichoderma koningii* 'TSG', and *Trichoderma asperellum* 'TH-11' strains at 20 mg/l and 50 mg/l

concentrations in the medium, and at 70 mg/l the maximum productivity was also shown by *Trichoderma koningii* 'TSG' strain.

Meanwhile, high concentration of both mononitrotoluene and phenol 70 mg/l inhibited the growth of *Trichoderma harzianum* 'Mg-6' and *Trichoderma koningii* 'TSL-06' strains. The negative effect of high concentrations in the case of mononitrotoluene was found still on the growth of *Trichoderma asperellum* strain 'TH-11', in the case of phenol on that of *Trichoderma asperellum* 'M99/5'.

We used depth culturing to evaluate the ability of strains of the genus *Trichoderma* to degrade mononitrotoluene and phenol. The deep culture method involves growing microorganisms in a liquid nutrient medium under periodic stirring. The microbial cells grow in the entire volume of the liquid nutrient medium and in suspension. This method provides the possibility of intensive mycelial growth, accumulation of metabolic products, and a high level of process mechanisation. However, it does not provide a complete development cycle for mycelial fungi. The sporulation stage in these conditions in phenotype is poorly expressed or is not performed at all [20]. Therefore, we used Chapek's nutrient medium without the addition of agar. After sterilisation we cooled the flasks with liquid medium, inoculated the strains using a microbiological loop, and took the results on the 14th day.

According to analysis results, investigated strains of the genus *Trichoderma* are able to degrade mononitrotoluene and phenol contained in the medium to different degrees.

When MNT was added to the medium at a concentration of 20 mg/l, the strains Trichoderma koningii 'TSG', Trichoderma harzianum 'Mg-6', *Trichoderma koningii* 'TSL-06' showed the greatest degree of degradation. They reduced its concentrations almost twice – by 48%, 47%, and 41.5%, respectively.

Trichoderma asperellum strains 'TH-11' showed the greatest reduction of MNT *concentration* by 42% and *Trichoderma koningii* strains 'TSL-06' showed the greatest reduction of MNT concentration by 40.6%.

Addition of mononitrotoluene to the medium at a concentration of 70 mg/l showed the highest degree of degradation of *Trichoderma koningii* strain 'TSL-06' with a 66% decrease in concentration. The strains *Trichoderma asperellum* 'TH-11' and *Trichoderma koningii* 'TSG' also reduced its concentration by 47% and 38%, respectively (Fig. 1.).

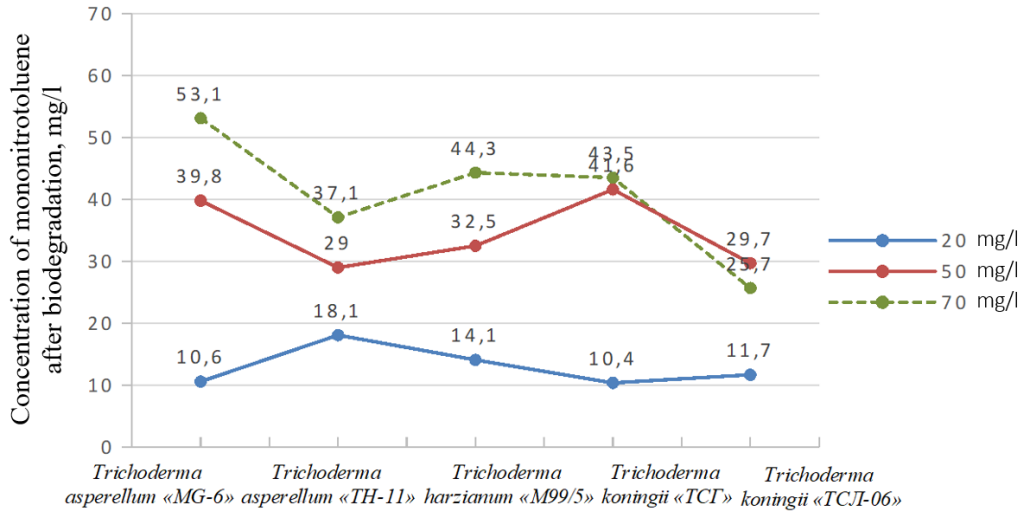

Fig. 1. Results of mononitrotoluene biodegradation at different concentrations in the medium by strains of the genus *Trichoderma*

The highest reduction of phenol concentration in the medium at 20 mg/l was shown by *Trichoderma harzianum* strain 'M99/5' and the concentration decreased by 30%. *Trichoderma asperellum* strain 'Mg-6' and *Trichoderma koningii* strain 'TSL-06' decreased the concentration by 17.5 and 15%, respectively; Trichoderma asperellum strain 'TH-11' and *Trichoderma koningii* strain 'TSG' decreased the concentration by less than 9%.

The highest biodegradation efficiency compared to other initial concentrations was characteristic of phenol content in the medium of 50 mg/l. There was a significant decrease in its concentration by *Trichoderma harzianum* strain 'M99/5' by 95.5%; *Trichoderma asperellum* strain 'Mg-6' and *Trichoderma asperellum* strain 'TH-11' decreased its concentration by 46.6 and 42%, respectively; *Trichoderma koningii* strains 'TSL-06' and *Trichoderma koningii* strain 'TSG' - by 39%.

The strains Trichoderma asperellum 'TH-11', *Trichoderma asperellum* 'Mg-6' and *Trichoderma harzianum* 'M99/5' decreased at 70 mg/l *phenol* concentration its concentration in the medium by 17.2; 14.3, and 12.9 %, respectively; the strains *Trichoderma koningii* 'TSL-06' and *Trichoderma koningii* 'TSG' - by 11.4 % (Fig. 2).

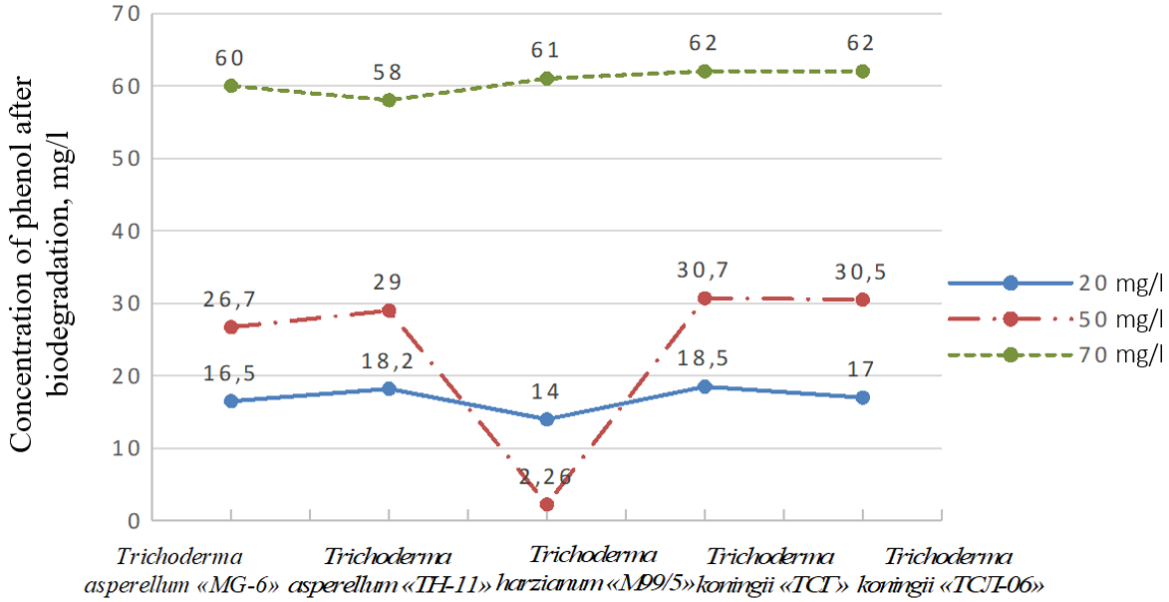

Fig. 2. Results of phenol biodegradation at different concentrations in the medium by strains of the genus *Trichoderma*

Indeed, high concentrations of aromatic compounds (70 mg/l) had a toxic effect on the growth of *Trichoderma harzianum* 'Mg-6', *Trichoderma koningii* 'TSL-06', *Trichoderma asperellum* 'TH-11' and *Trichoderma asperellum* 'M99/5' strains during surface cultivation. However, at deep cultivation these strains showed the greatest destruction of toxicants.

Trichoderma koningii strain 'TSG' showed the greatest resistance to mononitrotoluene and phenol in the medium among all the *studied* micromycetes. Reduction of its productivity under conditions of surface cultivation was not observed at all concentrations. This strain was the only one able to grow and develop in the presence of high concentration of 70 mg/l of the tested chemicals. However, the strain was less effective in deep cultivation.

Conclusions and recommendations

According to research results, fungi of the genus *Trichoderma* are able to grow in the presence of such toxic industrial pollutants as mononitrotoluene and phenol, but have different sensitivity to changes in their concentration in the environment. The highest productivity was observed at xenobiotics content in the range of 20-50 mg/l. The only strain of *Trichoderma koningii* 'TSG' was resistant to high content of pollutants in the medium.

The strain Trichoderma koningii 'TSG' can be recommended for treatment of *wastewater* containing mononitrotoluene and phenol in conditions of surface cultivation for pollutants up to 70 mg/l, and strains *Trichoderma asperellum* 'TH-11' and Trichoderma harzianum 'M99/5' can be recommended for pollutants up to 50 mg/l.

Fungi of the genus *Trichoderma* are able to degrade mononitrotoluene and phenol in waters. Decomposition of mononitrotoluene under the action of micromycetes was more intensive under conditions of deep cultivation at its high concentration in the medium of 70 mg/l. The maximum efficiency was 66% (*Trichoderma koningii* strain 'TSL-06'). The maximum degradation of phenol was 95% at 50 mg/l (*Trichoderma harzianum* strain 'M99/5').

We can recommend Trichoderma koningii 'TSG' and *Trichoderma koningii* 'TSL-06' strains to produce a biopreparation on the basis of *micromycetes* for treatment of industrial wastewater for removal of mononitrotoluene at combining surface and deep cultivation principles in one immersion biotechnological system. We recommend *Trichoderma koningii* 'TSG' and *Trichoderma harzianum* 'M99/5' for phenolic water treatment.

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Received 05.09.2024
Approved 19.09.2024
Accepted 23.09.2024