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POSSIBILITIES OF USING LIGNINOLYTIC FUNGI FOR BIOLOGICAL WASTE GAS TREATMENT

Possibilities of using wood destroying fungi (*Phanerochaete chrysosporium* and *Trametes versicolor*) for the removal of some selected organic pollutants from gases were investigated. The following compounds were used: toluene, chlorobenzene, tetrachloromethane, aniline, butyl alcohol and ethyl acetate. The experiments were performed in two types of purification systems: in a laboratory biofilter filled with a mixture of straw and peat and inoculated with a mycelium, and in a bioreactor containing a fungal biomass immobilized on polyethylene support and filled either with a nutrient-rich (growth) medium or a nitrogen-insufficient (induction) medium. The depletion of toluene and chlorobenzene was not observed in the biofilter. In the bioreactor filled with induction medium, *Phanerochaete chrysosporium* degraded chlorobenzene at the rate of 1.7 mg/m^3·s. This system also removed tetrachloromethane from waste gases. At low inlet concentrations of this pollutant (120 mg/m^3) the removal efficiency reached 100%, but at high concentrations (1720–2680 mg/m^3) the efficiency approached 40%, and a maximum elimination capacity of 2.25 mg/m^3·s was found. The best performance of biofilter was observed when butyl alcohol was degraded by *Phanerochaete chrysosporium* (elimination capacity 7.35 mg/ m^3·s, removal efficiency 93.8%). The removal of aniline from waste gases in biofilter with *Trametes versicolor* also occurred (elimination capacity 1 mg/m^3·s, removal efficiency of 74–96%).

1. INTRODUCTION

Biological waste gas treatment is an alternative to classic physicochemical techniques. This method is based on two elementary processes: sorption of contaminants and their subsequent degradation by microorganisms. A sorbent may be solid (compost, peat) or liquid (activated sludge, culture medium), and sorption and biodegradation processes may proceed in the same place (biofilters, bubble columns) or may be spatially separated (bioscrubbers).

A biological method has two important advantages:

- it allows us to save energy, because biological processes take place at normal, contrary to common physicochemical ones (thermal incineration), temperature and pressure [1],

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• it is practically waste-free, because sorbent self-regeneration takes place owing to biological mineralization of pollutants, which is an advantage over the adsorption techniques limited by adsorbents' capacity [1], [2].

In biological methods, the indigenous microorganisms present in the sorbent used are active agents. These are mainly bacteria and, to a lesser extent, the so-called lower fungi (moulds and yeast). However, some researchers pay attention to the advantages given by the biofilter bed inoculated with fungi, especially in treating gases contaminated by hydrophobic pollutants [3]. It appears that these organisms are tolerant of low bed humidity. When the water layer around the mycelium is reduced, the mass transfer rate of these compounds can be improved. Also, the strongly branched aerial hyphae of fungi form large surface area that is in direct contact with the contaminants in gas stream, and in such a case an aqueous phase does not take part in the process. One group of higher fungi produce peroxidases and oxidases of a high substrate specifity and therefore they have a unique ability to degrade lignin. These organisms in the mid-1980 attracted growing attention of biologists and sanitary engineers dealing with environmental protection, because it has been proven that the ligninolytic activity is associated with ability to biodegrade and also to mineralize a wide variety of persistent environmental pollutants [4]. Special attention of researchers has been focused on one group of fungi that in nature cause the so-called white rot of wood. These fungi belong to different orders representing *Basidiomycotina, Basidiomycetes*, but most of them are from the *Aphyllophorales* order. The ligninolytic enzymes are synthesized and secreted extracellularly in response to such a trigger factor as a low concentration of some pivotal nutrients, e.g. carbon, nitrogen or sulfur (starvation, i.e. ligninolytic, conditions) [4]. Mechanisms like this are independent of the occurrence of substrate in the surroundings of mycelium and therefore, in contrast to bacterial systems, the substrate acclimation is not required [4]. The *Trametes versicolor* species produces not only lignin peroxidases, but *p*-phenol oxidase, called laccase. Production of laccase may be stimulated either by starvation or by some compounds like phenols and aromatic amines [5].

The white-rot fungi are already applied in wastewater treatment, especially in the case of such pollutants as chlorophenols and trinitrotoluene (TNT), and in bioremediation of soils contaminated by aromatic hydrocarbons, TNT and pentachlorophenol (PCP). These fungi are also used in pulp and paper industry to remove lignin from wood pulp, enabling reduction of the amount of chlorine compounds usually used in the process [6]–[8]. But papers about the application of the white-rot fungi to waste gas treatment are very few. MAJCHERCZYK et al. [9] investigated the removal of styrene in a biofilter packed out with chopped straw grown over with mycelium of selected strains of white-rot fungi. He reported that high concentrations of styrene (1245 mg/m^3) resulted in its high removal efficiency (95–100%). Similar results were also observed for H_2S and NH_3. But the authors did not give any additional information about technological parameters of the process (e.g. biofilter loading), which would be useful to a better appreciation of the results. BRAUN-LULLEMAN et al. [10] investigated the ability of different strains of ligninolytic fungi to

remove some organic pollutants, e.g. toluene, xylenes, ethylbenzene, styrene and aniline, from the gaseous phase. But these experiments, except the styrene removal, were carried out in simple static systems, where gas was not passed gas through the bed.

The aim of our experiment was to verify the usefulness of white-rot fungi for waste-gas treatment in the conditions of gas flowing through the purification system. Two best known species from this group of fungi were used: *Phanerochaete chrysosporium* and *Trametes versicolor*. The experiments were carried out in two model systems: in a lab-scale biofilter and in a reactor with a biomass immobilized on inert support. The systems differed in the water phase content and in the specific surface area of mass transfer. Biofilters are characterized by low water content in the bed and a large gas–biofilm exchange area, they are especially suitable for the removal of hydrophobic pollutants [3]. In our investigation, the laboratory biofilters were filled with chopped wheat straw mixed with peat. This kind of bed is conducive to induction of ligninolylic enzymes because of a low nitrogen content. On the other hand, in the bioreactors with immobilized fungal biomass, the purification proceeded in a liquid phase, where a specific exchange area between gas and liquid was small [3]. In these bioreactors, the growth (full) medium or the induction medium (with low nitrogen content) was used as a liquid phase. In the liquid medium, the fungal biomass immobilized on polyethylene support was dipped. Such xenobiotics as toluene, chlorobenzene, tetrachloromethane, aniline, butyl alcohol and ethyl acetate were selected as model pollutants, which differed in their chemical structure and water solubility.

2. MATERIALS AND METHODS

2.1. FUNGI AND INOCULUM

Two strains of white-rot fungi were used in the experiments: *Phanerochaete chrysosporium* Burdsall., FCL 92, FPL and *Trametes versicolor* (L. ex Fr.) Pil., FPD, FCL 20. The strains were donated to us by the Fungal Culture Collection of Biochemistry Department of the Maria Curie-Skłodowska University in Lublin. They were stored on 2% malt agar slants at 278 K (5 °C).

In order to obtain the *Phanerochaete chrysosporium* inoculum, the 3–4-week-old slants were flooded with sterile distilled water. The prepared suspension of conidia and mycelium hyphae was poured on Petri dishes with malt extract agar. The dishes were incubated at 312 K (39 °C) during 7 days and next the agar discs overgrown with mycelium were impressed and introduced into a liquid medium with malt extract. After 7-day incubation the grown mycelium was disintegrated in glass homogenizer. The suspension obtained was used as inoculum for both types of the systems tested.

The inoculum of *Trametes versicolor* was prepared by transferring a piece of mycelium to 100 cm^3 of growth medium according to FÅHRAEUS [11] in 1000 cm^3 Erlenmeyer

flask. After 7-day incubation at 298 K (25 °C) the mycelia formed were disintegrated in glass homogenizer. The suspension obtained was used as inoculum of biofilter bed.

2.2. LABORATORY-SCALE BIOFILTER

A simplified scheme of the laboratory installation and a principle of its operation are presented in figure 1. The biofilter (*6*) was made of PCV pipe, 1.2 m long and 200 mm of inside diameter, which was filled with three layers of bed, each of 300 mm high (*7a*, *7b*, *7c*). The bed was composed of wheat straw mixed with acid peat in a ratio of 1:1 (volume). This mixture was sterilized in an autoclave, and next inoculated with mycelium (1% by weight). The bed humidity was fixed at 45% of the wet mass using mineral medium, which contained the following components per 1 dm^3 of distilled water: KH_2PO_4, 2 g; $NaNO_3$, 0.2 g; $MgSO_4$, 0.5 g; $CaSO_4$, 0.1 g; mineral solution, 5 cm^3; thiamine, 5 cm^3. The air flow sucked from the room by membrane pump (*1b*) was humidified in dumping column (*4*) made of PCV pipe, 1.00 m long and 100 mm of inside diameter, filled with wet active carbon up to 0.90 m. Using another membrane pump (*1a*) the air flow was sucked and passed through the bubbler (*3*) filled with the pollutant tested in order to obtain its saturated vapour. After mixing of both streams in the mixing chamber (*5*), the gas mixture entered the biofilter (*6*).

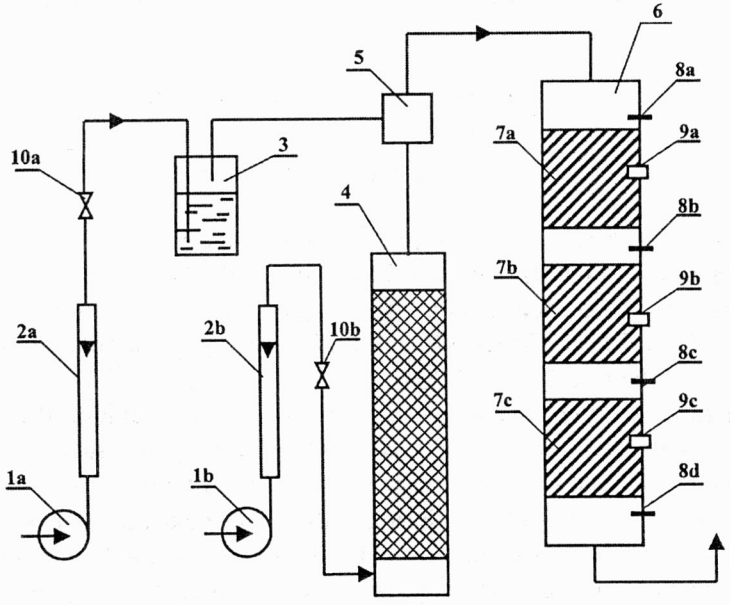

Fig. 1. The laboratory-scale biofiltration system used: *1a*, *1b* – membrane pumps, *2a*, *2b* – rotameters, *3* – bubbler saturating air with pollutant, *4* – air damping column, *5* – mixing chamber, *6* – biofilter, *7a*, *7b*, *7c* – layers of filter bed, *8a*, *8b*, *8c*, *8d* – pipe connectors for gas sampling, *9a*, *9b*, *9c* – pipe connectors controlling filter bed moisture, *10a*, *10b* – gas flow control valves

Operation parameters and overall dimensions of the biofilter were as follows:

- temperature — ca. 293 K (20 °C),
- volume of the filter column (V) — $7.07 \cdot 10^{-3}$ m^3 (for *Phanerochaete*), $2.5 \cdot 10^{-3}$ m^3 (for *Trametes*),
- cross-section field of the filter bed — $7.85 \cdot 10^{-3}$ m^2,
- flow rate of gas volume (Q) — 0.5 m$^3 \cdot$ h^{-1} (for *Phanerochaete*), 0.2 m$^3 \cdot$ h^{-1} (for *Trametes*),
- volumetric load of the filter column (Q/V) — 70.7 h^{-1} (for *Phanerochaete*), 80 h^{-1} (for *Trametes*).

2.3. LAB-SCALE BIOREACTOR WITH IMMOBILIZED BIOMASS

A simplified scheme of the bioreactor is presented in figure 2. Its main part was a glass pipe, 1.50 m long and 25 mm of inside diameter (*6*). The inside of the pipe was lined with polyethylene net (*7*), 2 mm thick and 6 mm net mesh. The net was a support for fungal biomass. In order to immobilize mycelium, the biomass homogenate and growth medium were sterilely introduced into the two pipes. The growth medium contained the following components per 1 dm^3 of distilled water: glucose, 10 g; malt extract, 10 g; peptone, 2 g; yeast extract, 2 g; asparagine, 1 g; KH_2PO_4, 2 g; $MgSO_4 \cdot 7H_2O$, 1 g; thiamine, 1 mg. At first the pipes were kept in horizontal position, aerated and periodically turned about their long axis in order to obtain the uniform overgrowth of the support by mycelium. After 7 days of incubation at a room temperature, the bioreactors were placed in vertical position and then they were filled up with growth medium. Thereafter they were incubated for 7 days. Then, the medium was removed and replaced with fresh growth medium (exchanged every several days) or with induction medium in order to induce the production of ligninolytic enzymes by mycelinum. The induction medium according to KIRK [12] was used. This medium contained the following components per 1 dm^3 of distilled water: glucose, 0.5 g; KH_2PO_4, 2 g; $NaNO_3$, 15 mg; $MgSO_4$, 0.5 g; $CuSO_4$, 0.1 g; minerals, 5 cm^3; thiamine, 5 mg; veratryl alcohol (10 mM), 5 cm^3. The air flow sucked by membrane pump (*1*) was saturated with vapours of the pollutant tested in the bubbler (*2*). The filter made of sterile glass wool (*5*) protected the bioreactor against microbial contamination.

Operation regime and overall dimensions of the bioreactor were as follows:

- working temperature — ca. 293 K (20 °C),
- volume of the bioreactor column (V) — $7.36 \cdot 10^{-4}$m^3,
- surface of the biological film ($2\pi rh$) (as an area of a cylinder) — 0.12 m^2,
- flow rate of gas volume (Q) — $7 \cdot 10^{-3}$m$^3 \cdot$ h^{-1},
- volumetric load of the bioreactor(Q/V) — 9.51 h^{-1}.

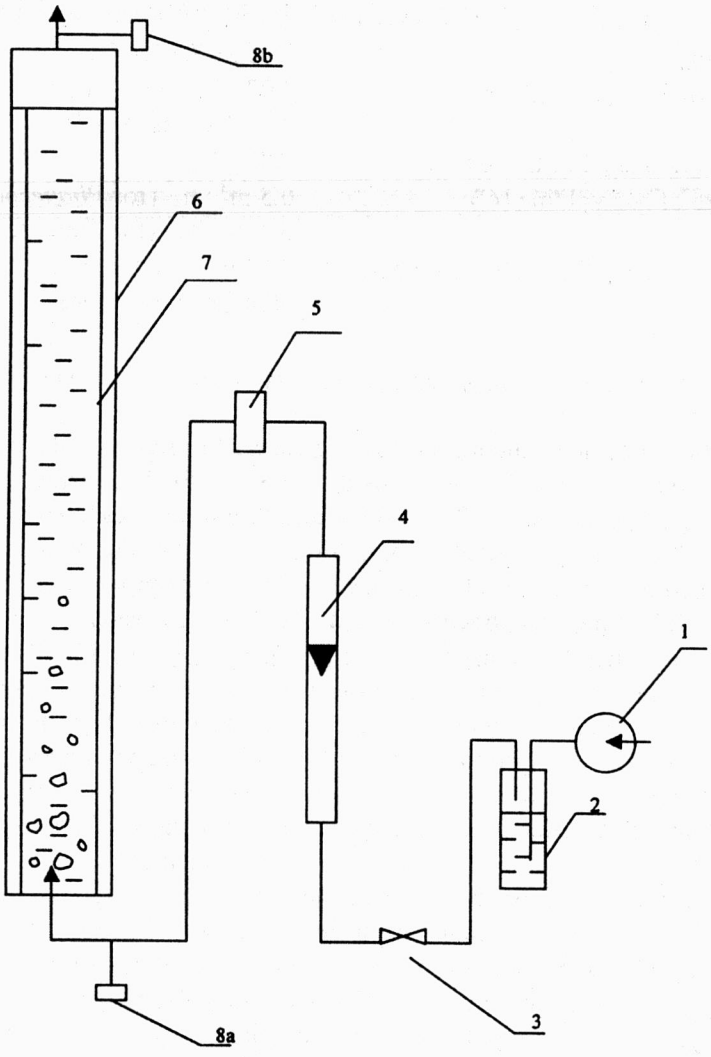

Fig. 2. The laboratory-scale bioreactor used: *1* – membrane pump, *2* – bubbler saturating air with pollutant, *3* – gas flow control valve, *4* – rotameter, *5* – filter made of sterile glass wool, *6* – bioreactor, *7* – polyethylene net with immobilized biomass, *8a*, *8b* – gas sampling points

2.4. CHROMATOGRAPHIC ANALYSIS

The following compounds were investigated: toluene, chlorobenzene, butyl alcohol, ethyl acetate (in the biofilter with *Phanerochaete*), aniline (in the biofilter with *Trametes*), and tetrachloromethane and chlorobenzene (in the bioreactor with *Phanerochaete*).

Concentrations of the pollutants tested were determined using the GCHF 18.3 type gas chromatograph. The inlet and the outlet concentrations (C_{in}, C_{out}) were measured. They were calculated based on the area of specific peak by means of the ACCORD computer program. Operation parameters of the chromatograph were as follows:

- flame – ionization detector (FID),
- carrier gas: nitrogen,
- steel column 1 m long, 4 mm dia., filled with carbowax,
- column temperature: 393 K,
- sample injector temperature: 453 K,
- detector temperature: 473 K.

The data obtained were used to calculate:

- The inlet load L

$$L = \frac{Q(\text{flow rate of gas volume}) \cdot C_{in}}{V \cdot 3600} \text{ [mg/m}^3 \cdot \text{s]}.$$

- The elimination capacity EC, the amount of a pollutant being removed in the biofilter or bioreactor

$$EC = \frac{Q(\text{flow rate of gas volume}) \times (C_{in} - C_{out})}{V(\text{volume of column}) \times 3600} \text{ [mg/m}^3 \cdot \text{s]}.$$

- The removal efficiency, i.e. fraction of pollutant (E) entering the biofilter or bioreactor which is removed from the air stream, typically expressed as per cent:

$$RE = \frac{C_{in} - C_{out}}{C_{in}} \cdot 100 \text{ [\%]}.$$

3. RESULTS AND DISCUSSION

3.1. AROMATIC HYDROCARBONS

Though the inlet toluene load of the biofilter bed was low (2 mg/m^3 · s), no purification effect was observed during 7-day passing the toluene vapours through this biofilter with *Phanerochaete chrysosporium*. BRAUN-LULLEMAN at al. [10] obtained analogous results to ours, but on a smaller scale. They investigated the possibilities of toluene removal from the gaseous phase using the mycelium of *Phanerochaete chrysosporium* growing on straw in tightly closed 10 cm^3 bottles, and they did not find any change in toluene concentration after 48 h of incubation. No removal of this compound, or slight removal, the authors also observed in the case of another well-known white-rot fungi

like *Pleurotus ostreatus*, *Bjerkandera adusta* and *Trametes versicolor*. On the other hand, YADAV and REDDY [13] stated that *Phanerochaete chrysosporium* effectively degraded not only toluene, but also all BTEX compounds both under ligninolytic conditions and (especially) on carbon and nitrogen-rich culture medium, so under conditions when the synthesis of ligninolytic enzymes is suppressed. These authors estimate that the biodegradation activity of the fungus studied is high. However, if we recalculate the values reported by them, it appears that 1 g of dry weight of mycelium, in optimum circumstances, degrades no more than 0.5 mg of toluene per day. Assuming that the biomass content in biofilter bed used in our experiments was comparable with that in the culture described by YADAV and REDDY (170 mg of dry weight/50 cm^3) and taking into account the operation parameters and dimensions of the biofilter, we may say that the activity mentioned above is far insufficient to obtain the detectable purification effects, even if the inlet concentration of toluene (C_{in} = 100 mg/dm^3) and inlet load (L = 2 $mg/m^3 \cdot s$) are low. In such a case, only 0.2 mg of toluene would be removed during 1 s by 1 m^3 of a filter bed. Therefore, our finding that the toluene vapours are not removed in biofilter can be confirmed (directly and indirectly) by both of the publications cited.

3.2. CHLORINATED HYDROCARBONS

Analogically, chlorobenzene was not degraded in the same biofiltration system with *Phanerochaete chrysosporium*. But its concentration was reduced in the bioreactor with mycelium immobilized on polyethylene support, both on growth medium and on induction medium (tables 1 and 2). In the growth medium system, the highest rate of the chlorobenzene removal approached 0.55 $mg/m^3 \cdot s$ at the inlet load L = 1.9 $mg/m^3 \cdot s$ (table 1). The removal efficiency within the examined concentration range was ca 30%. Application of the induction medium gave better results (table 2). In this case, the highest rate of chlorobenzene elimination capacity EC = 0.85 $mg/m^3 \cdot s$ was found at the inlet load L = 1.7 $mg/m^3 \cdot s$, which was equivalent to the removal efficiency E = 52%. This system, in contrast with the former one, showed the differentiation of chlorobenzene removal efficiency, which reached the highest value (E = 67%) at the inlet load L = 0.75 $mg/m^3 \cdot s$.

The elimination capacity and removal efficiency in the bioreactor with induction medium were enhanced by the activity of ligninolytic enzymes. Mycelium induced by nitrogen-starvation conditions is capable of producing such enzymes. But YADAV and co-workers [14], investigating of chlorobenzene biodegradation by *Phanerochaete chrysosporium*, found a reverse correlation. According to these authors, the rate of chlorobenzene removal on the growth medium was higher than on the induction one. The authors investigated the changes in chlorobenzene concentration in static culture growing in tightly closed Erlenmeyer flasks (v = 100 dm^3), so under different conditions than those in bioreactors described here. They found that ligninolytic enzymes did not participate in the chlorobenzene biodegradation, but this conclusion was not supported by investigation of enzymatic activity of culture medium. In addition to

this, CHEN et al. [15] reported that the ligninase production was possible under non-induction medium conditions. It may explain the activity of chlorobenzene depletion system on the growth medium described in this paper and in the mentioned paper of Yadav and coworkers.

Table 1

Removal of chlorobenzene in the bioreactor with the mycelium of *Phanerochaete chrysosporium* immobilized on polyethylene support in growth medium.
Flow rate of gas volume $Q = 7 \cdot 10^{-3} m^3/h$, volume of reactor $V = 7.36 \cdot 10^{-4} m^3$

Inlet concentration C_{in} [mg/m^3]	Outlet concentration C_{out} [mg/m^3]	$\Delta_c (C_{in} - C_{out})$ [mg/m^3]	Inlet load L [mg/m^3· s]	Elimination capacity EC [mg/m^3· s]	Removal efficiency E [%]
350	235	115	0.90	0.30	32.9
370	265	105	1.00	0.30	28.4
555	425	130	1.45	0.35	23.5
605	425	180	1.60	0.50	29.8
730	520	210	1.90	0.55	28.7

Table 2

Removal of chlorobenzene in the bioreactor with the mycelium of *Phanerochaete chrysosporium* immobilized on polyethylene support in induction medium.
Flow rate of gas volume $Q = 7 \cdot 10^{-3} m^3/h$, volume of reactor $V = 7.36 \cdot 10^{-4} m^3$

Inlet concentration C_{in} [mg/m^3]	Outlet concentration C_{out} [mg/m^3]	$\Delta_c (C_{in} - C_{out})$ [mg/m^3]	Inlet load L [mg/m^3· s]	Elimination capacity EC [mg/m^3· s]	Removal efficiency E [%]
180	100	80	0.50	0.20	45.3
285	95	190	0.75	0.50	67.1
635	305	330	1.70	0.85	51.8

The bioreactor with induction medium, which was characterized by higher elimination capacity of chlorobenzene than the reactor with growth medium, was used in tetrachloromethane (CCl_4) treatment investigation. The results obtained were shown in table 3. As can be seen when inlet concentrations of CCl_4 were low ($C_{in} = 120$ mg/m^3) and inlet load also was small ($L = 0.30$ mg/m^3 · s), the removal efficiency of the system reached 100%. An increase in the inlet concentration caused a decrease in the removal efficiency, but the purification efficiency approached 31.7% even if the load of the bioreactor was relatively high ($L = 7$ mg/m^3 · s). Tetrachloromethane is extremely resistant to biodegradation in aerobic conditions because of its high oxidation number. On the other hand, anaerobic biodegradation is incomplete and results in accumulation of such toxic metabolites as dichloroethene (DCE) and $CHCl_3$ or CH_2Cl_2 [16]. The *Phanerochaete chrysosporium* fungus belongs to a small group of organisms that are able to complete mineralization of this hazardous com-

Table 3

Removal of tetrachloromethane in the bioreactor with the mycelium of *Phanerochaete chrysosporium* immobilized on polyethylene support in induction medium.
Flow rate of gas volume $Q = 7 \cdot 10^{-3} m^3/h$, volume of reactor $V = 7.36 \cdot 10^{-4} m^3$

Inlet concentration C_{in} [mg/m^3]	Outlet concentration C_{out}[mg/m^3]	$\Delta_c (C_{in} - C_{out})$ [mg/m^3]	Inlet load L [mg/m^3· s]	Elimination capacity EC [mg/m^3· s]	Removal efficiency E [%]
120	0	120	0.30	0.30	100
1720	1035	685	4.55	1.80	39.9
2680	1830	850	7.05	2.25	31.7

pound. KHINDARIA et al. [16], who investigated a mechanism of CCl_4 mineralization by this fungus, found that it was a reductive mechanism dependent on the ligninolytic system. This system produces the cation radicals of veratryl alcohol which are oxidized by peroxidase. These radicals, in turn, oxidize oxalates – the secondary metabolites produced by mycelium under starvation conditions. Next, the oxidized oxalate anion radicals are electron donors in reactions of reduction and dehalogenation of CCl_4. The products of these reactions, being devoid of chlorine, are of lesser toxicity and can finally be mineralized [4], [16].

3.3. ANILINE

Removal of aniline from waste gases was investigated in biofilter with bed composed of straw and peat and inoculated with *Trametes versicolor.* The results obtained are presented in table 4. As can be seen during the 13-day investigation the elimination capacity was steady and reached ca. 1 mg/m^3 · s at an inlet concentration of 50 mg/m^3. It was accompanied by a high removal efficiency approaching 90%. In the 18th day of investigation, when the inlet concentration of aniline was lower (C_{in} = 28 mg/m^3), a distinct decrease in both elimination capacity and removal efficiency was recorded. During the investigation the progressive blackening of the bed was observed. This phenomenon may be explained by the accumulation of polymerized products of reactions catalyzed by laccase – the main enzyme produced by *Trametes versicolor.* This enzyme catalyzes reactions associated with generating radical derivatives, which subsequently initiate polymerization reactions [17]. Accumulation of reaction products in the bed caused an increase of flow resistance and a decrease of inlet concentration. It also hampered the mycelium activity. For this reason, after 18 days of gas flowing through the biofilter, the elimination capacity and removal efficiency dropped to 0.47 mg/m^3·s and 74%, respectively. The results obtained prove that the bed inoculated with *Trametes versicolor* is effective in aniline removal, that in a purification system, the reaction products should be separated from the active biomass (by rinsing the biomass, for example).

Table 4

Removal of aniline in the biofilter filled with the wheat–peat bed overgrown with *Trametes versicolor*. Flow rate of gas volume $Q = 0.2$ m^3/h, volume of filter bed $V = 2.5 \cdot 10^{-3}$ m^3

Day	Inlet concentration C_{in}[mg/m^3]	Outlet concentration C_{out}[mg/m^3]	$\Delta_c (C_{in} - C_{out})$ [mg/m^3]	Inlet load L [mg/m^3· s]	Elimination capacity EC [mg/m^3·s]	Removal efficiency E [%]
4th	50	2.3	47.7	1.13	1.08	95.4
5th	50	2	48	1.13	1.09	96
6th	50	4	46	1.13	1.04	92
13th	48	6	42	1.09	0.95	87.5
18th	28	7.2	20.8	0.63	0.47	74.3
19th	28	7.2	20.8	0.63	0.47	74.3

Also BRAUN-LULLEMANN et al. [10] investigated the removal of aniline from gaseous phase by *Trametes versicolor*. They measured the drop in concentration of this compound caused by mycelium growing on straw in a closed system, without gas flow. They used a very high initial concentration of aniline (15.4 g/m^3) and after 48 h of incubation, they found that this concentration was reduced by 84%. This proved that the elimination capacity of the biofilter was 0.75 mg/m^3 · s, i.e. of the same order of magnitude as ours.

3.4. OXYGEN ORGANIC COMPOUNDS

The biofilter with the bed composed of straw and peat and grown over with mycelium of *Phanerochaete chrysosporium* was used. The results obtained are presented in tables 5 and 6. Of all pollutants examined, butyl alcohol and ethyl acetate appeared to be the most susceptible to biofiltration. This is caused by the high hydrophilicity of these compounds. The best results were obtained for the removal of butyl alcohol, whose removal efficiency proved to be high, even if the inlet load was relatively high (table 5). These data are similar to those obtained for conventional bacterial biofilters, which also purify gases of undesirable alcohols and esters most efficiently [18].

Table 5

Removal of butyl alcohol in the biofilter filled with the wheat–peat bed overgrown with *Phanerochaete chrysosporium*.
Flow rate of gas volume $Q = 0.5$ m^3/h, volume of filter bed $V = 7.07 \cdot 10^{-3}$ m^3

Inlet concentration C_{in} [mg/m^3]	Outlet concentration C_{out}[mg/m^3]	$\Delta_c (C_{in} - C_{out})$ [mg/m^3]	Inlet load L [mg/m^3· s]	Elimination capacity EC [mg/m^3· s]	Removal efficiency E [%]
85	60	25	1.70	0.50	29.4
350	50	300	6.90	5.90	85.7
400	25	375	7.85	7.35	93.8
420	40	380	8.25	7.45	90.5

Table 6

Removal of ethyl acetate in the biofilter filled with the wheat–peat bed overgrown with *Phanerochaete chrysosporium*.
Flow rate of gas volume $Q = 0.5$ m^3/h, volume of filter bed $V = 7.07 \cdot 10^{-3}$ m^3

Inlet concentration C_{in} [mg/m^3]	Outlet concentration C_{out} [mg/m^3]	$\Delta_c (C_{in} - C_{out})$ [mg/m^3]	Inlet load L [mg/m^3 · s]	Elimination capacity EC [mg/m^3 · s]	Removal efficiency E [%]
75	50	25	1.45	0.50	33.5
80	60	20	1.55	0.40	25
85	60	25	1.65	0.50	29.4
85	55	30	1.65	0.60	35.3
170	115	55	3.35	1.10	32.4
190	130	60	3.75	1.20	31.6
240	180	60	4.70	1.10	25.0
255	165	90	5.00	1.75	35.3
265	200	65	5.20	1.30	24.5
335	245	90	6.60	1.75	26.5

4. CONCLUSIONS

The biofilter with *Phanerochaete chrysosporium* removed only hydrophilic contaminants from gases, like alcohols and esters, but was ineffective in removing aromatic pollutants. However, the latter compounds were removed in the bioreactor with induction medium, where the fungus was under starvation conditions. Hence, it seems that this fungus may be used for waste gas treatment, provided that chemical composition of the medium can be controlled in order to induce and maintain the ligninolytic conditions. This points to the necessity of increasing the share of aqueous phase in a purification system, which means the application of such a device as, for example, a bioscrubber. In the purification system proposed, the enzymatically active culture fluid on an induction medium would act as a sorbent, and the regeneration of the sorbent would proceed in a separate bioreactor containing immobilized biomass. It appears that the ability of fungi to be active in the conditions of low humidity is not very important in the case of white-rot fungi. Although the concentration of nitrogen in the wheat–peat bed applied to the laboratory biofilter is low, such a bed is not effective in gas purification. It is not a simple matter to manipulate and control this system, therefore a conventional biofilter is not suitable for appllying *Phanerochaete chrysosporium*.

Although in the biofilter system, the efficiency of aniline removal from gases is high, it appears that such a system is not suitable either for *Trametes versicolor*. In the case of this fungus, the difficulty does not lies in inducing mycelium to enzyme production (in this instance, a substrate itself may be a inductor), but in accumulating polymerized reaction products in the bed. This increases the flow resistance of gases

and isolates the mycelium. Low stability of laccase – the main enzyme produced by *Trametes versicolor*, presents another problem. Therefore, the share of an aqueous phase in the purification process seems such advantageous as in the case of *Phanerochaete chrysosporium.* It would allow us to separate the reaction products from the active biomass. Besides this, LUTEREK et al. [17] found that laccase stability may be clearly enhanced by immobilization of the enzyme on inert support, e.g. on porous glass beads.

The important question connected with the application of white-rot fungi in waste gas treatment (and in bioremediation in general) is the operational stability of purification systems. In our investigation, any system did not operate longer than 19 days. Other authors also described investigations which lasted only a few days, or they did not reported how long their experiments lasted [9], [10]. The duration of experiment may be of special importance when the gases contaminated by microorganisms capable of suppressing the domination of a one fungal strain enter the bioreactor with the fungal monoculture. In the paper presented, before entering the purification system, the gases were passed through the filter composed of sterile glass wool. But the problem needs investigation, because sterilization of gas stream on a technological scale does not seem to be feasible.

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MOŻLIWOŚCI WYKORZYSTANIA GRZYBÓW LIGNINOLITYCZNYCH DO BIOLOGICZNEGO OCZYSZCZANIA GAZÓW

Zbadano możliwość wykorzystania grzybów rozkładających drewno (*Phanerochaete chrysosporium* i *Teametes versicolor*) do oczyszczania gazów z wybranych zanieczyszczeń organicznych (toluenu, chlorobenzenu, tetrachlorometanu, aniliny, butanolu, octanu etylu). Badania wykonano w dwóch typach układów oczyszczających: w biofiltrze laboratoryjnym wypełnionym mieszanką słomy i torfu, zaszczepioną grzybnią, oraz w bioreaktorze z biomasą immobilizowaną na nośniku polietylenowym, wypełnionym pożywką bogatą w składniki odżywcze (wzrostową) lub o niskiej zawartości azotu (indukcyjną). Biofiltr nie usuwał toluenu i chlorobenzenu, podczas gdy bioreaktor z *Phanerochaete chrysosporium* na pożywce indukcyjnej usuwał chlorobenzen z szybkością 1,7 mg/m^3·s. Układ ten oczyszczał również gaz z tetrachlorometanu. Gdy stężenia początkowe tetrachlorometanu były małe (120 mg/m^3), stwierdzono 100% skuteczność oczyszczania, natomiast gdy stężeni były duże (1720–2680 mg/m^3), skuteczność dochodziła do 40%, a maksymalna szybkość oczyszczania wynosiła 2,25 mg/m^3·s. W biofiltrze najefektywniej butanol usuwał grzyb *Phanerochaete chrysosporium* (szybkość oczyszczania 7,35 mg/m^3·s, skuteczność 93,8%). Stwierdzono również oczyszczanie gazów z aniliny na złożu z *Trametes versicolor* (szybkość 1 mg/m^3·s, skuteczność 74–96%).