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Biofiltration of Waste Gas Containing Cyclohexanol, Cyclohexanone and Butanol

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Abstract: The aim of the study was to determine the efficiency of a biofilter treating waste gas containing a mixture of cyclohexanol (CHXOH), cyclohexanone (CHXO) and butanol (BL) and to assess the impact of the process parameters on the microorganisms and their enzymatic activities. The biofilter was packed with compost made from green waste mixed with compost made from municipal wastes and polyethylene carriers with immobilized biomass of bacteria. A linear correlation between pollutant loading rate and biofilter elimination capacity was obtained. At a hydraulic load of the biofilter bed of 127.3 $m^3/(m^3 \times h)$, the average elimination capacities were 14.6 g CHXOH/$(m^3 \times h)$, 3.6 g CHXO/$(m^3 \times h)$ and 3.8 g BL/$(m^3 \times h)$. The microbiological and enzymatic analyses of the biofilter bed indicated that high loading rates of pollutants can have significant effects on microbial growth and enzymatic activity.

Keywords: biofiltration; cyclohexanol; cyclohexanone; butanol; TTC-dehydrogenase activity; fluorescein diacetate hydrolase activity

1. Introduction

The development of efficient methods of waste gas treatment is one of the factors playing an important role in air protection and controlling air quality all over the world. Since the mid-twentieth century, biotechnological methods have been gradually gaining in popularity in the removal of gaseous pollutants from waste gases. These methods use the processes that occur in natural soil and water conditions. Polluted air is filtrated through soil and water and comes into contact with bacterial populations. The absorbed pollutants are then biotransformed by microorganisms which use them as a source of carbon and energy. The advantages are: the high efficiency in removing biodegradable substances, selectivity, low costs and the lack of waste products [1–5].

The most important factors in deciding to use biotechnological waste gas treatments are the bioavailability and biodegradability of the relevant air pollutants. The waste gases must also be free from dust and components that are toxic to microorganisms, and their temperature should not inhibit the metabolic activity of microorganisms involved in the biodegradation process [1,6].

The first bioreactor applied to treat waste gases from a wastewater treatment plant in the 1950s incorporated a biofilter. Since then, various bioreactor configurations have been applied to treat waste gases, including bioscrubbers, biotrickling filters, continuously stirred tank bioreactors, airlift bioscrubbers, dual liquid-phase systems, external-loop airlift bioreactors, membrane bioreactors, rotating drums and two-stage bioreactors [1,2,7–11]. However, until now, the classic biofilter remains the most common reactor used for biological waste gas treatment due to the easy operation, high efficiency and relatively low investment, maintenance and operation costs.

A biofilter is filled with natural organic and/or inorganic materials (the biofilter bed) which serve as carriers for microorganisms. Materials used in biofilter beds should be durable, chemically and physically stable, and have large specific surface areas. They

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should also have a low density and a high porosity (minimum 40–60%), have a high water-holding capacity and sorption capacity, and provide low gas-flow resistance. Typical materials used in biofilters include compost from municipal waste or green waste, bark, leaves, heather, brushwood, wood particles, branches and leaves, bark, peat, soil, dehydrated activated sludge, lava, marble or mixtures of these materials. Mixtures of natural materials with synthetic (e.g., polystyrene foam) or inert carriers (e.g., ceramics, perlite and glass beads) are also often used. The addition of synthetic carriers should ensure better porosity and lower gas-flow resistance through the biofilter bed. The advantage of using natural materials is the presence of macro- and microelements and moisture contents necessary for the growth of microorganisms [12–17].

Natural materials have one more advantage as biofilter beds. These materials are inhabited by a large variety of microorganisms. Apart from bacteria, there are also archaea and fungi active in the biodegradation of many different compounds, some of which can pose difficulties in typical biodegradation processes. Therefore, the additional inoculation of biofilters is not necessary, although in cases when gas pollutants are hardly biodegradable it may be feasible to inoculate the biofilter bed with specialized bacterial strains active in biodegrading these pollutants [18–21]. The bacteria and archaea detected in biofilters include, among others, Proteobacteria (α-, β-, γ- and δ-), Actinobacteria, Thermoprotei (Crenarchaeota), Firmicutes and Verrucomicrobia, such as *Rhodococcus* sp., *Mesorhizobium* sp., *Afipia* sp., *Nitrobacter* sp., *Devosia* sp., *Sphingomonas* sp., *Burkholderia* sp., *Methylophilales* sp., *Alcaligenes* sp., *Methylobacillus* sp., *Stenotrophomonas* sp., *Escherichia* sp., *Shigella* sp., *Enterobacter* sp., *Flexibacter* sp., *Pedobacter* sp., *Rhodobacter* sp., *Flavobacterium* sp., *Magnetospirillum* sp., *Hyphomicrobium* sp., *Thiobacillus* sp. and *Chitinophaga* sp. [2,21,22]. Fungi detected in biofilters treating hardly biodegradable compounds, such as monoterpenes or BTEX, belonged mostly to Basidiomycota (*Phanerochaete chrysosporium*) or Ascomycota (*Exophiala oligosperma, Exophiala lecanii-corni, Paecilomyces* sp., *Scedosporium apiospermum* and *Sporothrix variecibatus*) [2,23].

Different microbial taxa (bacteria, archaea and fungi) can grow in biofilters. Their growth and substrate utilization rates depend not only on the type of gas pollutant and carrier composition, but also on the operational parameters, such as waste gas temperature, substrate mass loading rate and volumetric loading rate. As a result, microbial communities in biofilters differ between even very similar biofilter configurations treating waste gases with the same main pollutants. Cho et al. [24] used a rock wool–compost biofilter inoculated with *Sphingomonas* sp. to treat waste gases polluted with ethylbenzene, o-xylene and BTEX at ambient air temperature, while a biofilter used to treat BTEX in thermophilic conditions was inhabited by Actinobacteria *Rubrobacter xylanophilus* and *Mycobacterium hassiacum* [24,25]. Wang et al. [26] also analyzed the mesophilic and thermophilic operations of a biofilter treating waste gas contaminated with toluene. The dominant organisms detected in the thermophilic biofilter were *Brevibacillus* sp. and *Anoxybacillus* sp., while *Delftia* sp. and *Stenotrophomonas* sp. dominated the biofilter operated in mesophilic conditions.

Since the 1980s, microbiological analyses of microbial communities within biofilters have been based on cultivation methods. However, these methods are usually limited mostly to one or several species that are able to grow on cultivating media. Considering the differences between microbial communities inhabiting even similar biofilter carriers observed over several years, molecular methods, such as quantitative real-time and reverse transcription (RT) polymerase chain reaction (PCR), semiquantitative denaturing gradient gel electrophoresis (DGGE), fluorescence in situ hybridization (FISH), microarrays (GeoChip 2.0/3.0, Phylo-Ch), clone libraries and sequencing, protein-/amino acid-SIP, FAME and others, are used to analyze the microbial communities in biofilter beds and to determine the dominant species and dominant gene sequences [2,23]. Li and Moe [27] used PCR-DGGE by amplification of coding gene 16 S rRNA. They proved that diversity in microbiological structure was a function of the spatial positioning of the bacteria at bioreactor height. Chung [28] conducted a phylogenetic analysis of microorganisms in a biofilter

filled with compost with the addition of activated carbon and activated sludge. He used PCR-DGGE and FISH techniques to determine changes in the bacterial community in the biofilter during the deodorization processes of gases derived from composting food waste. Phylogenetic analysis revealed that the predominant phylum was that of the Proteobacteria. Actinobacteria, Bacteroidetes and Firmicutes were also present in significant numbers.

Compared to routine methods for determining changes in bacterial counts in biofilters, Alvarez-Hornos et al. [29] proved that a better way to determine the impact of biofilter loading rate is to determine the dynamics of live and dead bacterial cells. A fluorescence microscope with the LIVE/DEAD BacLight™ Bacterial Viability Kit by Molecular Probes (Carlsbad, CA) was used to determine the numbers of live and dead bacterial cells in four biofilter sections. It was shown that the number of dead cells increased with increasing biofilter loading rate with ethyl acetate, toluene or a mixture thereof. This effect was best observed in the top section of the biofilter, where the concentration of pollutants was highest and where they had a toxic impact on microorganisms.

There are few data on the enzymatic activities of microorganisms inhabiting biofilters. Kan and Deshusses [30] used the 2-(p-iodo-phenyl)-3-(p-nitrophenyl)-s-phenyl tetrazolium chloride (INT) test to determine the biomass activity in a foamed emulsion bioreactor (FEBR) treating gas with toluene. It was proved that during 144 h of the process, without the addition of nutrients or cells, the toluene removal efficiency decreased from 90% to 64%, the biomass concentration by about 30% and the enzymatic activity by about 88%.

A literature review also indicated that most studies on the removal of VOCs from waste gases in biofilters focused either on individual compounds (mainly aromatic or chlorinated hydrocarbons) or on mixtures of pollutants containing mainly easily biodegradable VOCs (waste gases from composting or from wastewater treatment plants). However, no data are available on the biofiltration of waste gases containing cyclic aliphatic compounds, such as cyclohexanol or cyclohexanone. These compounds are widely used in the production of polymers and are therefore widely used, e.g., in the production of packaging, paints and insulation and in the varnish industry, among other contexts.

The aim of this study was to determine the biofiltration efficiency in the treatment of waste gas containing a mixture of cyclohexanol (CHXOH), cyclohexanone (CHXO) and butanol (BL) as the first step in developing technology to treat waste gas from the production of packaging for medicines and cosmetics from a factory in Poland and to assess the impacts of the process parameters on the microorganisms and their enzymatic activities, including TTC-dehydrogenase and hydrolase activity.

Cyclohexanol (CHXOH) is a cyclic alcohol with the general chemical formula $C_6H_{11}OH$ [30]. Its most important use is the oxidation of cyclohexanol to adipic acid in the manufacture of nylon-6,6. The next most important use of cyclohexanol, pure or mixed with cyclohexanone as KA-oil, is in the production of caprolactam, which is used in the manufacture of nylon-6 polymer. Lesser amounts of this compound are also used as solvents for alkyd resins, alcohol-soluble phenolic resins, ethyl cellulose—especially in the paint industry and paint and varnish production—and in the manufacture of celluloid, finishing textiles and insecticides [31].

Cyclohexanone (CHXO) is a cyclic organic ketone with the general chemical formula $C_6H_{10}O$. Similar to cyclohexanol, cyclohexanone is used as an intermediate in polymer production and in chemical technology and as a solvent in the paint industry and in the production of dyes, pigments, lacquers and varnish [32].

The presence of both cyclohexanol and cyclohexanone in the air has a significant impact on human life and health. The effect of inhaling vapors of these compounds is irritation of the skin, eyes, nose and throat. Both chemicals can also have a narcotic effect, affecting the human nervous system. Cyclohexanone is a weak central nervous depressant, while exposure to cyclohexanol can result in narcosis-depression of the central nervous system, sleep and unconsciousness. Longer exposure to air contaminated with cyclohexanol and cyclohexanone may cause permanent damage to the liver and kidneys [33].

Cyclohexanol and cyclohexanone are produced commercially by the catalytic air oxidation of cyclohexane or the catalytic hydrogenation of phenol. CHXO may be also prepared by either the catalytic dehydrogenation or oxidative dehydration of cyclohexanol. The oxidation of cyclohexane to a mixture of CHXOH and CHXO, known as KA-oil (ketone–alcohol, cyclohexanone–cyclohexanol crude mixture) is nowadays used in most KA-oil production processes, despite its low yield of 6–8% [31,34]. Alternative production pathways need to be developed for an economically viable production process, hence the search for biocatalysts, such as microorganisms that are able to degrade cyclohexane to cyclohexanol and cyclohexanone under environmentally benign non-toxic operation conditions [34]. Research by Salamanca et al. [35–37] and Karande et al. [38] indicated that cyclohexane can be degraded to cyclohexanol and cyclohexanone and further to caprolactone by cyclohexanol dehydrogenase and cyclohexanone monooxygenases.

Butanol (butyl alcohol—BL) is an alcohol with the chemical formula C_4H_9OH [39]. It is used in the chemical industry as an intermediate element in the production of glycol ethers, herbicides and derivatives of butyl alcohols; in the textile industry as a solvent in the production of polyvinyl; and in the automotive industry as an element of brake fluids. In the pharmaceutical industry, butanol is used to extract antibiotics, vitamins and hormones. Inhaling butanol vapors irritates the skin, eyes, nose and throat; this compound also affects the human nervous system, causing weakness, dizziness, vomiting, depression and delays in mental reactions [33,39].

2. Materials and Methods

2.1. Biofilter Set-Up

A schematic diagram of the experimental set-up is shown in Figure 1. The compressed air stream was introduced into the system by an air pump and then divided into two parts. One part was passed through the RTU- 06-160 rotameter (Zakłady Automatyki Rotametr Sp. z o.o., Gliwice, Poland) and the bubbler vessel containing a mixture of cyclohexanol, cyclohexanone and butanol 5:13:2 (v/v). Both streams were then connected and entered the mixing tank. The prepared waste gas was additionally humidified by a water vapor humidifier (Warsaw University of Technology, Warsaw, Poland). Then, after passing the rotameter, the waste gas entered the biofilter, which was operated in upflow mode.

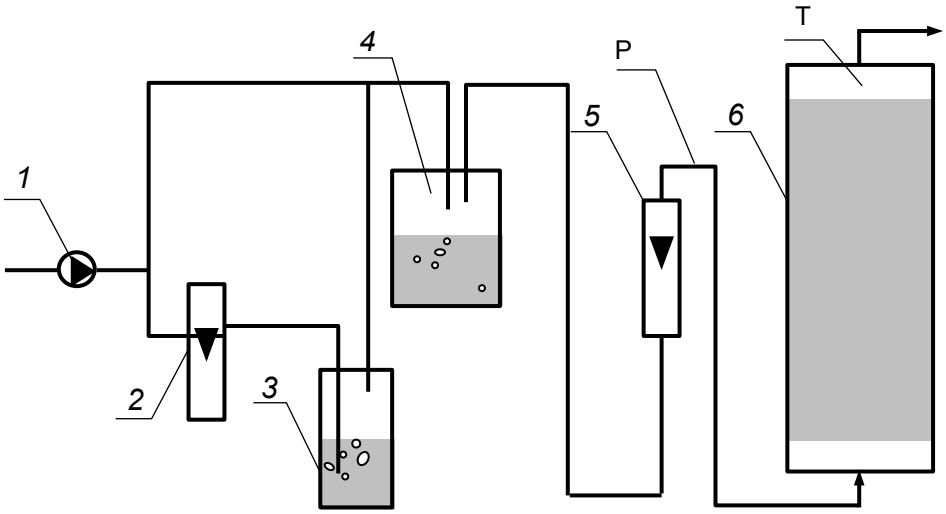

Figure 1. Biofilter experimental installation. 1—air pump, 2—rotameter with air valve, 3—bubbler vessel, 4—mixing tank with humidifier (water), 5—rotameter, 6—biofilter, P—sample point of polluted air, T—sample point of treated air.

The biofilter was made of plexiglass (PMMA) and had a height of 1.2 m and an inner diameter of 0.2 m. The height of the biofilter bed was 1 m, and its working volume was 0.0314 m^3. The biofilter was packed with compost made from green waste mixed with

compost made from municipal wastes and polyethylene carriers with immobilized biomass of bacteria (3:2:1 *v*/*v*). Moisture (measured on a wet basis) was maintained at 50–80% by periodically spraying the filter bed with water.

The biofilter operated under the following conditions: the average hydraulic load of the biofilter in the first stage of the experiment was equal to 95.5 $m^3/(m^3 \times h)$ and in the second and third stages of the experiment to 127.3 $m^3/(m^3 \times h)$. Waste gas humidity was 100%, and the temperature was 20–24 °C. The average pollutant loading rates in all stages of the research are presented in Table 1.

Table 1. Average pollutant loading rates during the experiment.

Pollutants	Loading Rates of Pollutants ($g/(m^3 \times h)$)		
	1st Stage of Experiment	2nd Stage of Experiment	3rd Stage of Experiment
Cyclohexanol (CHXOH)	56.8	8.8	14.4
Cyclohexanone (CHXO)	27.3	1.7	4.8
Butanol (BL)	30.9	2.0	5.2

The technological parameters set in the first stage of the experiment were based on theoretical research on waste gas composition for such types of production (data not shown). The poor solubility in water and the high volatility of the main gas pollutants were taken into account when choosing the value of the average hydraulic load in the first stage of the experiment. In the second and third stages of the experiment, average hydraulic load was increased, while the concentrations of pollutants were decreased to correspond to real pollutant emissions.

Each stage of the experiment was continued until steady-state conditions were achieved for 3 weeks. All analyses were performed twice a week in triplicate.

2.2. Gas Sampling and Analysis

A sample of 100 cm^3 of air for gas chromatography analysis was collected using a Pocket Pump 210-1002TX (SKC). Air was taken from the sampling point and was passed through a glass tube filled with activated carbon Anasorb CSC. A sampling point of polluted air was situated between the rotameter and the biofilter inlet. Samples of treated air were taken at the biofilter outlet. Air pollutants were then desorbed from activated carbon using 1 cm^3 CS2. A quantity of 1 µL of prepared solution was introduced into the GC injection port manually.

Cyclohexanol, cyclohexanone and butanol concentrations in the contaminated air were estimated using a Hewlett Packard GC 5890A gas chromatograph (Hewlett Packard, Wilmington, DE, USA) equipped with a HP-5 column Crosslinked 5% PH ME Siloxane column (30 m × 0.53 mm × 1.5 pm; HP part no. 19095J- 323, USA) and operated with an injector temperature of 180 °C and a detector (FID) temperature of 270 °C. The initial oven temperature of 40 °C was held for 4 min and then increased to 60 °C at a rate of 1 °C/min. Helium (6 cm^3/min) was used as a carrier gas.

Air temperature and humidity were measured with the LB-701 thermometer–hygrometer with the LB-702 control panel (LAB-EL, Reguły, Poland). Air-flow velocity in the system was measured with an ROL-164 B rotameter. The pressure drop caused by the biofilter bed was measured with a pressure gauge.

2.3. Microbiological Sampling and Analyses

Samples of the biofilter bed for the microbiological and enzymatic analyses were taken from the sampling ports situated 25 cm and 75 cm from the bottom; these were described as the 'bottom layer' and the 'top layer', respectively. Suspensions of bacteria were prepared by shaking 10 g of carrier or biofilter bed with 90 cm^3 of sterile sodium pyrophosphate (0.1% solution) for 30 min at 120 rpm.

The numbers of bacteria active in contaminant biodegradation were determined in accordance with the European Standard PN EN ISO 6222:2002 by plating on mineral media with pollutants as the sole sources of carbon. The concentrations of pollutants were 0.5 g/dm^3. Organic compounds were added to the mineral solutions in the form of water emulsions obtained after treatment by ultrasonic waves (35 kHz). Colonies of heterotrophic bacteria on mineral media with pollutants were counted after 7 days of incubation at 26 °C. The results were presented as numbers of colony-forming units per gram of dry weight of biofilter bed (cfu/g d.w.).

The dry mass and humidity content in the biofilter bed were measured by drying the sample in an oven at 105 °C in accordance with the Polish Standard PN-EN 12880:2004.

Dehydrogenase activity was measured in accordance with Polish Standard PN-C-04616-8 2008 using triphenyl tetrazolium chloride (TTC). Two kinds of sample—one without an exogenous source of energy and one with glucose as an additional source of carbon—were incubated for 24 h in a rotary shaker (120 rpm) at 26 °C. Specific enzyme activity was estimated in a spectrophotometer at 490 nm by measuring the concentration of triphenyl formazan (TF) formed.

Hydrolase activity was determined using a modified fluorescein diacetate (FDA) assay [40]. Samples containing a bacterial suspension in phosphate buffer (pH 7.6) and fluorescein diacetate at a concentration of 20 μg/cm^3 were incubated for 24 h in a rotary shaker (120 rpm) at 26 °C. The concentration of formed fluorescein was measured spectrophotometrically at 490 nm.

Protein concentration was determined by the Lowry method. Protein was assayed in a spectrophotometer (at 750 nm) in cell-free extracts obtained by ultrasonic disintegration.

2.4. Statistical Measures and Methods

Mood's median test was used to test the null hypothesis that the medians of two series from two different stages of the experiment would be identical. Pearson's chi-squared test was performed at a significance level of 0.05, corresponding to a χ^2_{crit} value of 3.8. The Student's *t*-test was applied to determine the correlations between biofilter loading rates and elimination capacities for different pollutants.

3. Results

The results of the studies on the elimination of CHXOH, CHXO and BL from the waste gas are presented in Figure 2. The efficiencies of pollutant removal from waste gas in steady-state conditions in all stages of the experiment were very high, regardless of pollutant loading rate or hydraulic load. CHXOH removal efficiency was in the range of 81–97%, BL was removed from waste gas with 78–97% efficiency, while CHXO removal efficiency varied from 75% to 96%. The elimination capacity of every pollutant increased with the increase in the biofilter loading rate of the pollutant. A correlation was found between the load of pollutant removed in the biofilter and the biofilter loading rate for the ranges of the biofilter loading rates that were analysed in the research. This was confirmed by significance tests for biofilter loading rates of 8.8–56.8 g/(m^3 × h) for cyclohexanol, 1.7–27.3 g/(m^3 × h) for cyclohexanone and 2.0–30.9 g/(m^3 × h) for butanol. These results can be described with the following formulae:

- For BL: EC = 0.9349 × L with R^2 = 0.997;
- For CHXOH: EC = 0.8808 × L with R^2 = 0.996;
- For CHXO: EC = 0.9202 × L with R^2 = 0.998;

where EC = elimination capacity and L = pollutant loading rate.

The flow resistance through the biofilter bed changed during the experiment. In the 1st stage of the experiment, when the average hydraulic load of the biofilter was 95.5 m^3/(m^3 × h), the pressure drop varied from 441 Pa/m to 686 Pa/m. In the 2nd and 3rd stages of the experiment, the pressure drop was in the range of 1352 to 1793 Pa/m.

During the research, the pH of the biofilter bed did not change, and it was in the range of 7.40–7.70.

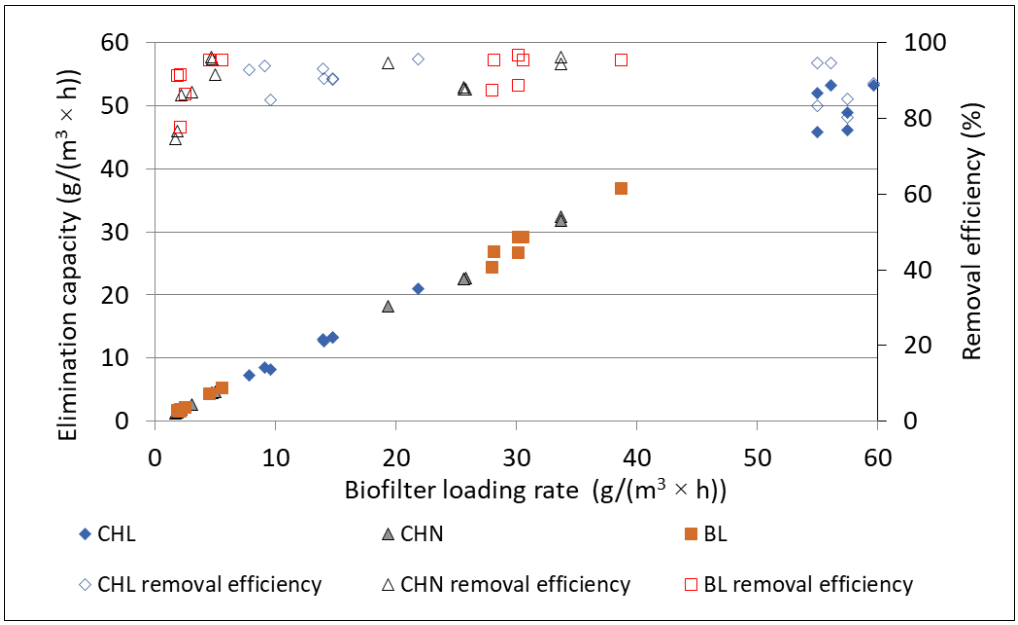

Figure 2. Elimination capacity vs. pollutant loading rate in the biofilter during the 1st, 2nd and 3rd stages of the experiment.

Microbiological analyses showed that throughout the duration of the research the number of bacteria active in CHXOH degradation varied during the experiment (Table 2). In the 1st stage, with the greatest CHXOH loading rate, the number of bacteria active in its biodegradation was the lowest (10^7–10^8 cfu/g d.w.). The highest number of bacteria active in CHXOH degradation (2.4×10^9 cfu/g d.w.) was observed in the 2nd stage of the experiment, with the lowest biofilter loading rate for CHXOH. It should be noted that there were no significant differences between the numbers of bacteria in the top and bottom layers in all stages of the experiment. However, significance tests confirmed the correlation between the biofilter loading rate with CHXOH and the number of microorganisms active in its biodegradation in both layers.

Table 2. The number of bacteria in different layers of the biofilter bed.

Bacteria	Layer of Biofilter Bed		Number of Bacteria (cfu/g d.w.)		
			1st Stage	2nd Stage	3rd Stage
Active in CHXOH degradation	Top	Median	7.7×10^7	2.4×10^9	1.7×10^8
		Range	$5.0–48 \times 10^7$	$8.4–24 \times 10^8$	$7.7–17 \times 10^7$
	Bottom	Median	5.1×10^7	1.1×10^9	3.6×10^8
		Range	$5.1–55 \times 10^7$	$1.3–110 \times 10^7$	$3.5–3.7 \times 10^8$
Active in CHXO degradation	Top	Median	3.3×10^8	1.3×10^{10}	3.1×10^8
		Range	$0.11–3.6 \times 10^8$	$1.2–13 \times 10^9$	$1.1–31 \times 10^7$
	Bottom	Median	1.8×10^7	8.3×10^9	4.4×10^8
		Range	$0–3.6 \times 10^8$	$1.1–8.3 \times 10^9$	$1.8–44 \times 10^7$
Active in BL degradation	Top	Median	2.6×10^8	1.2×10^{10}	7.3×10^8
		Range	$1.0–6.2 \times 10^8$	$8.3–120 \times 10^8$	$2.6–7.3 \times 10^8$
	Bottom	Median	1.0×10^8	1.1×10^9	5.8×10^8
		Range	$2–626 \times 10^6$	$4.0–11 \times 10^8$	$2–580 \times 10^6$

Similar observations were made for bacteria growing on mineral media with CHXO as the sole source of carbon and energy. Their highest number (1.3×10^{10} cfu/g d.w.)

was noted when the CHXO loading rate was lowest, and it dropped by two orders of magnitude when higher loading rates were applied. There were no significant differences between the numbers of bacteria in the top and bottom layers in the 2nd and 3rd stages of the experiment, but there was a significant difference between the medians for the top and bottom layers of the biofilter bed in the first stage.

The data obtained for the bacteria active in BL degradation indicated the same pattern as observed for CHXO and CHXOH. The lowest number of bacteria was observed in the first stage of the experiment, the highest (1.2×10^{10} cfu/g d.w.) in the 2nd stage. There was a significant difference between the median numbers of bacteria active in BL degradation in the top and bottom layers of the biofilter bed in the 2nd stage, with the higher number of bacteria being present in the top layer of the biofilter bed.

Contrary to the results of the microbiological analysis, the highest hydrolase activity was observed in the 1st stage of the experiment with the greatest pollutant loading rates (Figure 3). In the top layer of the biofilter bed, it was in the range of 0.39–0.95 mg of fluorescein/(kg of protein × s), and in the bottom layer it varied from 0.47 to 1.00 mg of fluorescein/(kg of protein × s). There were no statistically significant differences between the medians for the top and bottom layers in this stage of the experiment. There were, however, statistically significant differences between the hydrolase activities in the top and bottom layers in the 2nd and 3rd stages of the experiment. Hydrolase activities in the 2nd and 3rd stages in the top layer were similar (0.41–0.70 and 0.49–0.57 mg of fluorescein/(kg of protein × s), respectively) and both were significantly lower than in the 1st stage. In the bottom layer in the 2nd and 3rd stages, hydrolase activities were significantly lower than in the top layer in the corresponding stages and were in the ranges of 0.30–0.40 and 0.32–0.41 mg of fluorescein/(kg of protein × s), respectively.

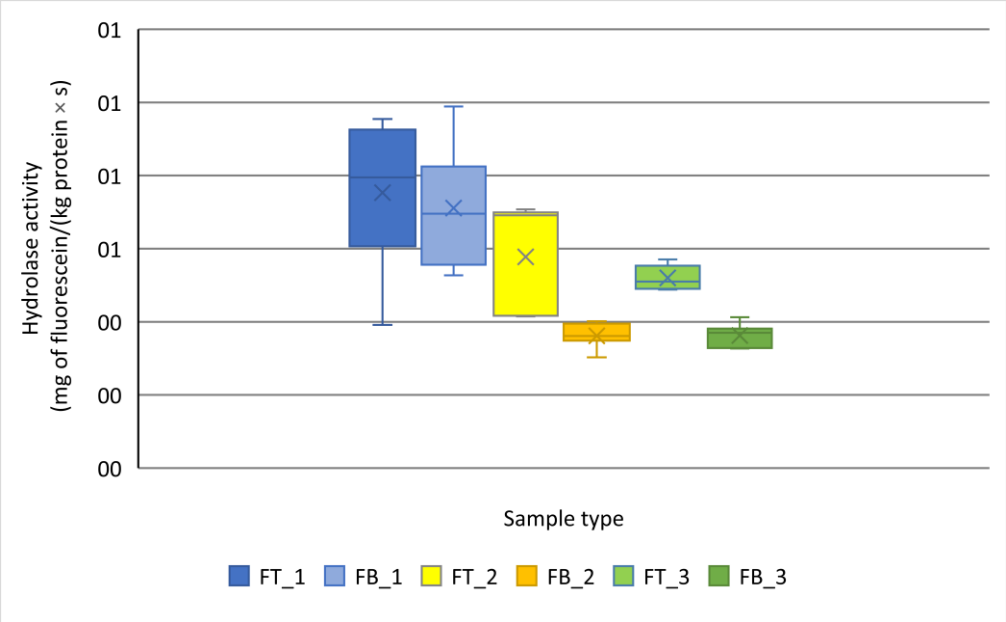

Figure 3. Hydrolase activity. F—fluorescein diacetate hydrolase; T—top layer, B—bottom layer, 1—1st stage of experiment, 2—2nd stage of experiment, 3—3rd stage of experiment.

In contrast to hydrolase activity, there was a significant difference between dehydrogenase activity (endogenous samples) in the top layer and in the bottom layer of the biofilter bed in the 1st stage of the experiment (Figure 4). The dehydrogenase activity in the top layer varied from 0.37 to 2.32 µmol TF/(kg of protein × s), while in the bottom layer it was in the range of 0.06–0.89 µmol TF/(kg of protein × s). Dehydrogenase activity in the top layer in the 1st stage of the experiment was also significantly higher than in the 2nd and 3rd stages of the experiment. However, there were no significant differences between the

dehydrogenase activities in the bottom layer between the 1st and 2nd and between the 1st and 3rd stages of the experiment and between the top layer and the bottom layer of the biofilter bed in the 2nd and 3rd stages.

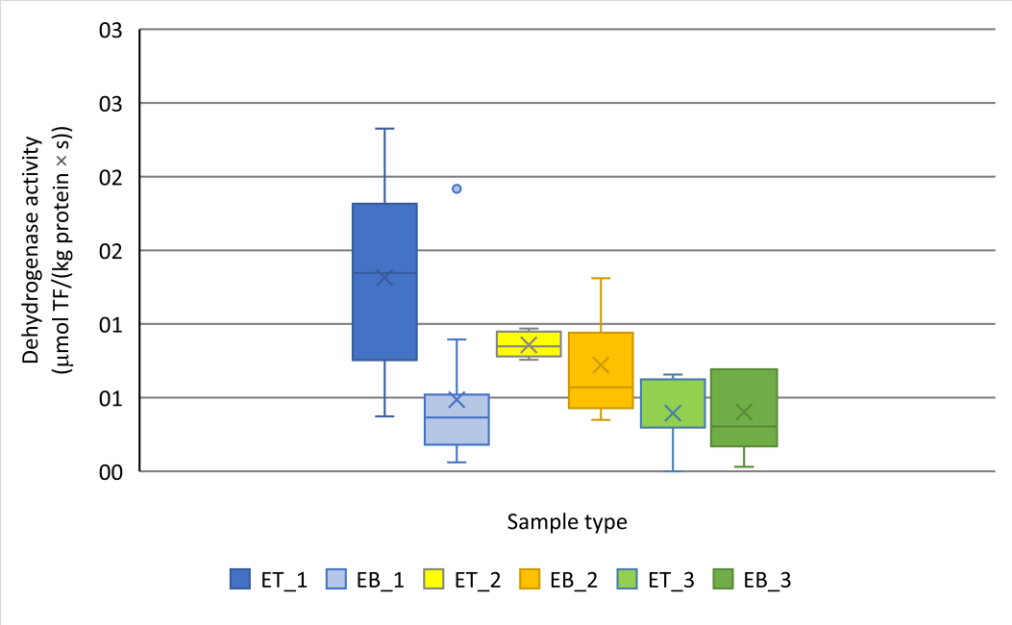

Figure 4. Dehydrogenase activity, endogenous samples. E—endogenous sample, T—top layer, B—bottom layer, 1—1st stage of experiment, 2—2nd stage of experiment, 3—3rd stage of experiment.

In the case of dehydrogenase activity, in samples with glucose (Figure 5), there were statistically significant differences in enzymatic activity only in the top layer between the 2nd and 3rd stages and between the 1st and 3rd stages of the experiment.

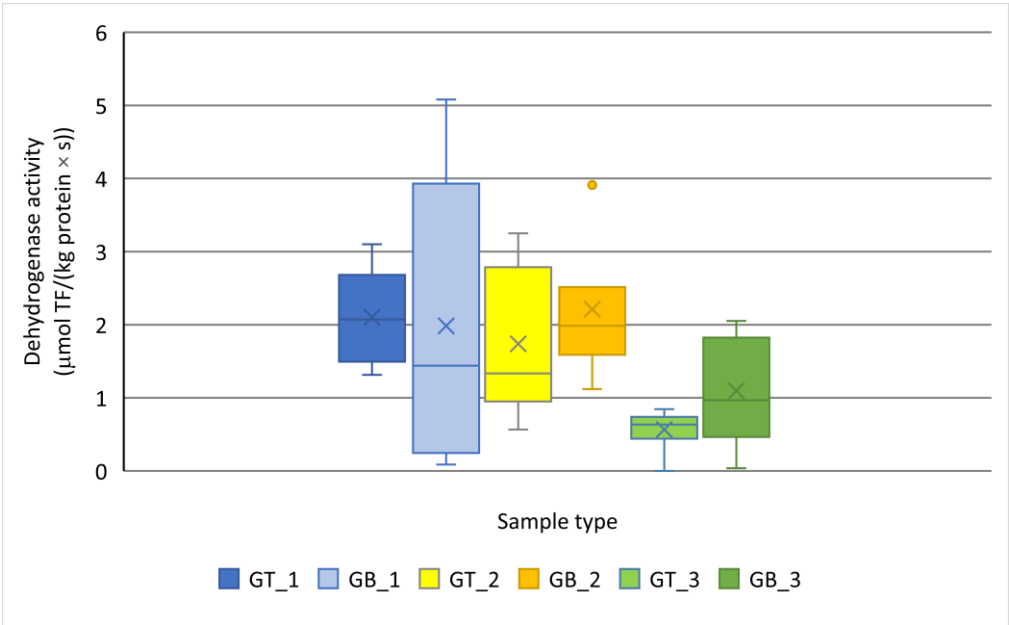

Figure 5. Dehydrogenase activity, samples with glucose. G—samples with glucose, T—top layer, B—bottom layer, 1—1st stage of experiment, 2—2nd stage of experiment, 3—3rd stage of experiment.

At the end of the study, the most effective bacterial strains with respect to the biodegradation of CHXOH, CHXO and BL were isolated and identified using API tests (Bio-Meriéux).

It was found that the biofilter was dominated by *Pseudomonas stutzeri* (50% of all detected bacterial strains) and two Gram-positive strains of *Micrococcus* (40% of all detected microorganisms). All three strains were capable of growing on mineral media with every type of pollutant.

4. Discussion

The average elimination capacities (ECs) of the biofilter in the first stage of the research were 52.2 g CHXOH/($m^3 \times h$), 24.4 g CHXO/($m^3 \times h$) and 28.3 g BL/($m^3 \times h$). In this stage, the biofilter operated under a very high loading rate and with quite a long empty bed residence time (EBRT) of 38 s. The obtained ECs were very high and exceeded the values typically observed and proposed for biofilter operations [1]. However, the efficiency measured in this stage was the lowest recorded in all the research (~75–80%). In the 2nd and 3rd stages, when the EBRT was shorter (28 s), the maximum ECs were 7.9 and 14.6 g CHXOH/($m^3 \times h$), 1.1 and 3.6 g CHXO/($m^3 \times h$), and 1.7 and 3.8 g BL/($m^3 \times h$), respectively. These observations agreed with research by Rene et al. [41], who analyzed the impact of pollutant load on the treatment of waste gas polluted with styrene in a perlite biofilter inoculated with the fungus *Sporothrix variecibatus.* The authors reported that the elimination capacity of the biofilter depended on the EBRT and decreased from 301 to 92 g/($m^3 \times h$) when the EBRT was changed from 91 s to 20 s.

The elimination capacities obtained in the 2nd and 3rd stages of the research were similar to the removed loads observed by Delhoménie et al. [42] in the biofiltration of waste air polluted with toluene, by Alvarez- Hornos et al. [29] for toluene and ethyl acetate, and by Paca et al. [43] for styrene. Analysis of the literature data concerning the elimination of other VOCs in biofilters, such as hexane and phenol, introduced into the biofilters at the same initial concentration [44] proved that the efficiency of the biofilter in this study was higher.

The maximum biofilter loading in the first stage of the experiment was 115 g of organics/($m^3 \times h$) or 2.7 kg /($m^3 \times d$). The average elimination capacity of the biofilter was 2.518 kg of organics/($m^3 \times d$). Theoretically, assuming that all organic pollutants were used as sources of carbon and energy by the microorganisms, with a maximum biomass yield value of 0.5, the actual amount of biomass growth should have been 1.26 kg/($m^3 \times d$) (~38 kg/m^3 within 30 days). Similarly, in the second stage, the maximum loading was 12.5 g of organics/($m^3 \times h$) or 0.3 kg /($m^3 \times d$), with an average elimination capacity of 0.26 kg of removed organics/(m3 $\times$ d). The biomass growth should have equaled 0.13 kg of biomass/($m^3 \times d$). However, microbiological analyses indicated that the numbers of bacteria active in BL, CHXOH and CHXO biodegradation were lowest in the 1st stage of the experiment and highest in the 2nd stage, when the pollutant loading rates were the highest and the lowest, respectively.

The negative correlation between the biofilter loading rate with CHXOH and the number of microorganisms active in its biodegradation was confirmed. These data confirm the observations made by Zilli et al. [45]. Zilli et al. [45] investigated a continuously operating bench-scale biofilter filled with a mixture of peat and glass beads and inoculated with the styrene-oxidizing strain *Rhodococcus rhodochrous*. The bioreactor was treating styrene-polluted gases. They proved that biomass development depended linearly on styrene concentration at a low inlet concentration. A maximum value of 7×10^7 cfu/g was achieved within a styrene concentration range of 0.10–1.0 g/m^3. Higher concentrations resulted in a rapid decrease in the number of microorganisms. The authors also confirmed the change in biomass concentration due to the change in pollutant concentration along the height of the biofilter bed.

In this study, there were no significant differences between the median numbers of bacteria active in contaminant biodegradation in the top and bottom layers of the biofilter bed, and they were usually in the same ranges in the same stages of the experiment. This observation, along with the decrease in bacterial numbers with the increase in pollutant loading rate, indicates that the pollutant concentrations applied in the study had effects

on biomass concentrations. Operating at different loading rates can result in changes to microbial communities and more efficient biomass (in terms of removing pollutants) could emerge in the biofilter.

Among the bacteria using BL, CHXOH and CHXO, *Pseudomonas stutzerii* and *Micrococcus* sp. were dominant. Dangel et al. [46] proved that denitrifying *Pseudomonas* species were able to metabolize cyclohexanol using the enzyme cyclohexanol dehydrogenase, which catalyzed the oxidation of the substrate to cyclohexanone. Cyclohexanone can be further oxidized by cyclohexanone dehydrogenase to 2- cyclohexanone or transformed by cyclohexanone monooxygenase to 1-oxa-2-oxocycloheptane. Further biotransformations catalyzed by dehydrogenases and lactonases can lead to the formation of adipate or caprolactone [34–38,47]. Cyclohexanone monooxygenase efficiently converts a variety of aliphatic, aromatic and cyclic ketones, as well as prochiral sulphides. This enzyme was observed in many bacterial strains and some fungi [48]. Butanol can be biodegraded by bacteria from the genus *Pseudomonas* to butyraldehyde and further to butyric acid by quinoprotein alcohol dehydrogenases and aldehyde dehydrogenase [49].

Several authors have analyzed the kinetics of the biotransformation of cyclohexanol and cyclohexanone to ε-caprolactone by cyclohexanone monooxygenase [50–53]. Tian et al. [51] investigated the activities of alcohol dehydrogenase (ADH) and cyclohexanone monooxygenase (CHMO) immobilized on the surface of *E. coli*. An apparent maximal reaction velocity (V_{MAX}(app)) for the oxidation of cyclohexanol with the ADH whole-cell biocatalysts was determined as 59.9 mU/mL of bacterial suspension. For the oxidation of cyclohexanone with the CHMO whole-cell biocatalysts, a V_{MAX}(app) of 491 mU/mL was obtained. Reimer et al. [52] noted that the actual enzymatic activity varied depending on whether the pure enzyme or whole bacterial cells were used. The uptake of cyclohexanol has been reported to be limited. A substrate uptake constant (K_S) of 3.57 mM by *Pseudomonas taiwanensis* was determined by Schäfer et al. [53].

In this study, the enzymatic activity (both hydrolase and dehydrogenase activity) in the biofilter bed was highest when the pollutant loading rate was the greatest. However, in the case of dehydrogenases, significant differences was observed between the top layer in the biofilter bed in the 1st stage of the experiment and in the 2nd and 3rd stages, and the enzymatic activity was significantly higher in the top layer than in the bottom layer, which may indicate inhibitory effects of the high loading rates of pollutants on the biomass in the bottom layer. There were no statistically significant differences in the median values for dehydrogenase activity between the two layers of the biofilter bed in the 2nd and 3rd stages of the experiment. There were also no statistically significant differences in the median values for hydrolase activity between the two layers of the biofilter bed.

García-Pẽna et al. [54] analyzed a biofiltration system inoculated with the mold *Paecilomyces variotii* treating waste gas polluted with toluene. Analyses of the activity of toluene oxygenase and benzylalcohol dehydrogenase proved a good agreement between the EC calculated from the enzymatic activity and the EC measured in the biofilter, suggesting that in the biofilters the EC was limited by the biological reactions. The data obtained in this study, however, did not prove a similar correlation.

5. Conclusions

Mixtures of butanol, cyclohexanol and cyclohexanone can be effectively removed from waste gas using a biofiltration process. For biofilter loading rates of 8.8–56.8 g/(m^3 × h) for cyclohexanol, 1.7–27.3 g/(m^3 × h) for cyclohexanone and 2.0–30.9 g/(m^3 × h) for butanol, the obtained results showed a linear correlation between pollutant loading rate and biofilter elimination capacity, despite the various hydraulic loads applied. However, the microbiological and enzymatic analyses of the biofilter bed indicated that a high loading rate of pollutants can have a significant impact on biomass concentration and dehydrogenase activity, thus indicating that lower loading rates should be applied at a technical scale.

At a hydraulic load of the biofilter bed of 127.3 $m^3/(m^3 \times h)$, the maximum elimination capacities were 14.6 g CHXOH/($m^3 \times h$), 3.6 g CHXO/($m^3 \times h$) and 3.8 g BL/($m^3 \times h$), with a pollutant removal efficiency of >90%.

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