Archives of Environmental Protection
Vol. 47 no. 1 pp. 26–34

PL ISSN 2083-4772
DOI 10.24425/aep.2021.136445

Removal of cyclohexane and ethanol from air in biotrickling filters inoculated with *Candida albicans* and *Candida subhashii*

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Keywords: biofilm, flow cytometry, removal efficiency, ethanol, biofiltration, cyclohexane

Abstract: This paper presents investigations on the removal of cyclohexane and ethanol from air in polyurethane-packed biotrickling filters, inoculated with *Candida albicans* and *Candida subhashii* fungal species. Results on process performance together with flow cytometry analyses of the biofilm formed over packing elements are presented and discussed. The results indicate that the presence of ethanol enhances the removal efficiency of cyclohexane from air. This synergistic effect may be attributed to both co-metabolism of cyclohexane with ethanol as well as increased sorption efficiency of cyclohexane to mineral salt medium in the presence of ethanol. Maximum elimination capacities of 89 g m^{-3} h^{-1} and 36.7 g m^{-3} h^{-1} were noted for cyclohexane and ethanol, respectively, when a mixture of these compounds was treated in a biofilter inoculated with *C. subhashii*. Results of flow cytometry analyses after 100 days of biofiltration revealed that about 91% and 88% of cells in biofilm remained actively dividing, respectively for *C. albicans* and *C. subhashii* species, indicating their good condition and ability to utilize cyclohexane and ethanol as a carbon source.

Introduction

The use of biofiltration processes to purify air from odorous compounds has been practiced for many years. Biofiltration, compared to other deodorization methods, has a number of advantages, such as low process costs, high purification efficiency for large volumes of gases with low odor concentrations, and very low emissions of secondary pollutants (Mudliar et al., 2010). A particularly attractive way of conducting biofiltration is the use of biotrickling filters. This is due to the possibility of quick adjustment of key process parameters (pH, composition and flow rate of the spraying liquid), which results in higher efficiency of air deodorization and longer life-time of such systems, compared to conventional biofilters (Rybarczyk et al., 2019a). In biotrickling filters, gas contaminated with odorous compounds is passed through a bed made of inert elements (e.g. polyurethane foam, ceramic elements), previously inoculated with consortium of microorganisms. The bed is trickled with liquid enriched with mineral salts. The trickling liquid circulates in a closed system, with the possibility of periodic replacement or regeneration. As a result of the growth of microorganisms, the so-called biofilm is formed over the packing elements. It is the biofilm in which compounds are adsorbed and absorbed from the gaseous phase and then undergo biodegradation.

It is well-known that efficient performance of biotrickling filters is greatly dependent on the biofilm formed over the packing elements (Purswani et al., 2011). What is more, microorganisms inhabiting the biofilm differ greatly in terms of both the rate of biofilm formation as well as ability to degrade pollutants (Feng et al., 2019). Usually, bacteria species are used for biofilter inoculation. Interestingly, fungi are known to be able to maintain the microbial activity under shock loads of pollutants, starvation periods or drought episodes (Rybarczyk et al., 2019b; Zhang et al., 2019). Besides, due to filamentous morphology, fungi offer a large surface area for, and thus increase the mass transfer of pollutants from the gas phase to the biofilm (Ferdowsi et al., 2017; Spigno et al., 2003). Because of the above listed features, fungi are of special interest when designing biotrickling filtration processes.

Biofiltration systems are especially efficient when water-soluble compounds are considered. Such compounds easily

break the mass transfer barrier between gaseous and aqueous (i.e. biofilm) phases and the rate of their biodegradation is mainly governed by the rate of biodegradation within the biofilm. Contrary, for poorly water-soluble compounds, i.e. hydrophobic ones, the efficiency of biofiltration depends greatly on the mass transfer rate between the above mentioned phases, and thus the biofiltration efficiency is much lower than for hydrophilic compounds (Cheng et al., 2016; Gospodarek et al., 2019). Several measures may be applied to improve the biofiltration performance with respect to hydrophobic compounds, including the addition of surfactants, especially biosurfactants, application of selected microbial species, including fungi, reactor modification, selection of proper process conditions as well as co-treatment with hydrophilic compounds (Cheng et al., 2020; He et al., 2020; Miller et al., 2019; Miller et al., 2020; Rybarczyk et al., 2020, 2019b; Yang et al., 2018, 2010).

In this paper, the possibility of using selected *Candida* fungi to simultaneously remove from air compounds with extremely different affinity to the aqueous phase was investigated. Hydrophobic cyclohexane and hydrophilic ethanol were used as model compounds. These compounds are found in post-processing gases from, e.g., paint, petroleum and food industries (Avalos Ramirez et al., 2007; Zhanga et al., 2018). Biotrickling filtration of air containing single cyclohexane or ethanol was previously investigated (Avalos Ramirez et al., 2007; Cox et al., 2001; Salamanca et al., 2017). In this paper, a mixture of cyclohexane and ethanol was subjected to biofiltration in two biotrickling filters, inoculated with *Candida albicans* and *Candida subhashii*, respectively. The composition of biofilms formed in two biotrickling filters was tested for purity of inhabiting fungi and compared between the process start-up and steady-state operation conditions using flow cytometry technique. To the best knowledge of authors of this paper, the above given fungi have not been used so far in biotrickling filters for air purification, and the search for new species of microorganisms capable of biodegradation of pollutants, especially of a hydrophobic nature, is an important trend in the environmental research.

Materials and methods

Investigations were performed on biotrickling filters made of plexi-glass columns of the following dimensions: 0.08 m in internal diameter and 0.68 m in height. Biofilters were packed with polyurethane foam discs (pore size PPI 10, Ultramare, Poland; dimensions of a single disc: 0.08 m in diameter, 0.01 m in height) up to the working volume of 2.5 dm^3 each. Biofilters were fed with a gas mixture from the bottom, while the trickling liquid was supplied from the top of a bioreactor, by means of a peristaltic pump. Gaseous mixtures of air with cyclohexane and ethanol (POCH, Poland) were obtained by passing the purified and dried air via a porous sinter through vials containing liquid cyclohexane and ethanol. The gas flow rate was controlled and regulated using a precise mass flow controller (Vögtlin, Switzerland). Gas flow rate of 2.5 dm^3 min^{-1} was used throughout the experiments, resulting in empty bed residence time (EBRT) equal to 1 min. Pressure drop across the packings of biotrickling filters was monitored using MPX5010dp sensors (NXP, the Netherlands) working in the range from 0 to 10 kPa. Maximum noted pressure did not exceed 2 kPa and no biomass overgrowth was observed throughout the experiments.

Gaseous samples containing cyclohexane and ethanol were taken from inlet and outlet gas streams. Samples were collected in Tedlar bags and concentrations of the above given volatile organic compounds were determined using gas chromatography technique using a DB-WAX column (30 m × 0.53 mm × 1 μm; Agilent Technologies, USA) and flame ionization detector (Varian CP-3800, VarianAnalytical Instruments, USA). Nitrogen was used as a carrier gas. The parameters of the analytical program were as follows: oven temperature: 100°C; FID detector temperature: 200°C, carrier gas flow rate: 3 cm^3 min^{-1}; split ratio: 10.

During the start-up period (first 7–10 days of biofiltration process), packing elements of biofilters were trickled with a Buffered Peptone Water medium (Merck, Germany). Then, mineral salt medium (MSM) was introduced. MSM contained the following salts dissolved in 1 dm^3 of distilled water: $Na_2HPO_4 \cdot 2H_2O$ (7.39 g), KH_2PO_4 (3 g), NaCl (0.5 g) and NH_4Cl (1 g) (POCH, Poland). Trickling liquid solutions were autoclaved before introducing to biofilters (Prestige Medical, England) and the MSM solution was exchanged once a week throughout the whole reported time period. Trickling liquid was sprayed over the packing elements with a frequency of 0.5 minutes per each hour, with a volumetric flow rate of 0.2 dm^3 min^{-1}.

Prior to the biofiltration start-up, packing elements made of polyurethane foam discs were inoculated with *Candida albicans* (biotrickling filter "A") and *Candida subhashii* (biotrickling filter "B"). Immobilization of fungi on polyurethane discs was realized using sterile beakers (each 1 dm^3 in volume) in which 600 cm^3 of Sabouraud medium (BTL, Poland) containing 10% (v/v) of selected fungi species inoculums was placed. These beakers were agitated in an orbital shaker (100 rpm, 24°C). After 24 hours of shaking, half of the medium volume was replaced with a fresh MSM solution. After the next 24 hours of shaking, the whole volume of the medium was replaced by a fresh portion of MSM solution. Then, a similar procedure was repeated, but MSM solution was replaced with Sabouraud medium in two steps as described above. The inoculation procedure lasted for 120 hours. Each day of inoculation, samples of media were taken and optical density measurements at wavelength of 595 nm using Thermo Scientific Multiskan FC spectrophotometer (Thermo Fisher Scientific, Finland) were routinely performed.

Two series of experiments were performed. In the first series (I), the performance of two biotrickling filters A and B was studied. Both biotrickling filters were initially fed with cyclohexane only, and at the 38th day of the process, ethanol was introduced into the gas stream. Additionally, flow cytometry analyses aiming at the determination of the general condition of microorganism populations inhabiting packing elements of biofilters were performed.

In the second series of experiments (II), one biotrickling filter inoculated with *C. subhashii* was investigated. Selection of these fungi species out of two investigated was done due to pathogenic characteristics of *C. albicans* as well as due to expected high performance of *C. subhashii* in the biotrickling filtration of hydrophobic volatile organic compounds. In this series, the biofilter was fed with a mixture of cyclohexane and ethanol from the process initiation. This approach was intended in order to study the effect of ethanol addition on the

performance of cyclohexane biofiltration, in comparison to experiments in series I. In series II, the liquid phase (MSM) was also investigated in terms of variations of pH as well as the concentrations of treated compounds during the biofiltration process.

The experimental staining technique with methylene blue (Sigma Aldrich, USA) was used to assess the formation of biofilm, containing the tested fungi, on the polyurethane foam elements. Photos of immobilized fungi were taken using transmitting light optical microscope with a 10× working distance lens (LAB 40 Series Optical Microscope, OPTA-TECH, Poland).

For cytometric analyses, single polyurethane discs were taken from each of the working biofilters. Each disc was placed in a beaker and suspended in 40 cm^3 of 0.01 M phosphate buffered saline solution (PBS, pH = 7.6) and shaken (4 times of 15 s shaking) in an ultrasonic bath (Bandelin Sonorex, Germany). After each shaking step, a beaker with a disk was placed in an water-ice bath for 15 s. The cell suspension was filtered using a 400-mesh nylon net to remove impurities. The precipitates were washed twice with PBS solution and suspended in 35 cm^3 of PBS solution after the centrifugation (6000 rpm, 6 min, 4°C; Eppendorf Centrifuge 5418R, Germany). A cell count was determined using a flow cytometry technique (Merck Millipore Guava easyCyte 8, Germany). A suspension volume containing 1 million of fungi cells was used in further investigations.

For the determination of microbial population condition using flow cytometry, 100 µL of AAB buffer (Annexin V Binding Buffer, BD Biosciences, Pharmingen, USA), 0.5 µL of FITC Annexin V (Annexin V fluorescein conjugate; Life Technologies Limited, Scotland) and 0.25 µL of 7-aminoactinomycin D (Sigma Aldrich, Germany) were added to the pellet. Staining with Annexin V was carried out for 15 minutes at 24°C in the dark. Finally, 100 µL of AAB buffer was added to the finished sample, in order to increase the sample volume. This conjugate can emit red and green fluorescence detected by fluorescence detector of flow cytometer.

For cytometric analyses of cell cycle of fungi, to the pellet were added: 300 µL of PBS, 300 µL of sodium deoxycholate (25 mM) (Sigma Aldrich, Germany) and 0.3 µL of propidium iodide, PI (2 mg cm^{-3}) (Sigma Aldrich, Germany). The final concentration of PI in the tubes was 1 mg/ml. Staining with propidium iodide was carried out for 30 minutes at 24°C in the dark. The measurement of fluorescence emitted by PI binded to DNA was performed using a flow cytometer. For propidium iodide, the wavelengths of excitation and emission are 488 and 617 nm, respectively. 10 000 of cells was analyzed during a single measurement.

The process conditions as well as performance of biotrickling filtration were described and evaluated using inlet loading (IL), empty bed residence time (EBRT), removal efficiency (RE) and elimination capacity (EC), according to the below given formulae:

$$IL = \frac{Q \cdot C_{in}}{V} \quad (1)$$

$$EBRT = \frac{V}{Q} \quad (2)$$

$$RE = \frac{C_{in} - C_{out}}{C_{in}} \quad (3)$$

$$EC = IL \cdot RE \quad (4)$$

where: Q – volumetric gas flow rate (m^3 h^{-1}), V – total volume of a biofilter packing (m^3), C_{in}, C_{out} – inlet and outlet concentrations of cyclohexane or ethanol in the gas stream (ppm v/v), respectively.

Results and discussion

Performance of biotrickling filtration using Candida albicans *and* Candida subhashii

In the first series of experiments, two biotrickling filtration processes were investigated. A mixture of air with cyclohexane and ethanol was treated either in a biofilter inoculated with *Candida albicans* (biofilter A) or *Candida subhashii* (biofilter B). Biotrickling filters were fed in a following manner. Initially (during the biofiltration start-up period), a gas stream contained only cyclohexane. Ethanol was added to the feeding gas stream after 38 days of biofiltration. During the first 10–14 days of biofiltration, the removal of cyclohexane from air is low. The results show that the removal efficiency of cyclohexane reaches the values of about 0.3–0.35 after the first 10–14 days of biofiltration (biofilter A, Fig. 1A). When biofilter B is concerned (Fig. 1B), values of RE fluctuate and do not exceed 0.25 during the first 30–35 days of bioflitration. A low removal efficiency is related to two main aspects: firstly, during the start-up period, the process runs under unsteady--state conditions when a biofilm formation precedes and the physico-chemical equilibria are set for the system. Secondly, the microbial flora inoculated onto the biofilters' packing elements undergoes acclimation to the treated compounds. The stabilization of the RE values, for both biotrickling filters, after about 14–38 days of the process duration indicate that steady--state conditions were attained since an increase of inlet loading at 21st day of biofiltration did not result in a decrease of the process performance.

At 38th day of biotrickling filtration processes, ethanol was added to the gas stream fed to the bioreactors. The addition of hydrophilic ethanol resulted in the enhancement of bioflitration performance, both for biofilters A and B (Figs. 1A and 1B). The removal efficiency of ethanol reaches about 99% after 45–50 days after the biofiltration process start-up. The values of RE, both for ethanol and cyclohexane, are slightly higher for the biotrickling filter A than B.

The increase of a process performance with respect to hydrophobic cyclohexane, upon the addition of hydrophilic ethanol, may be attributed to the promotion effect of the removal of hydrophobic compound. This effect may be a result of the stimulated growth and increased demand for carbon of microbial species inhabiting the biofilm (Cheng et al., 2020), leading to a co-metabolism of cyclohexane in the presence of ethanol. Moreover, the improved removal efficiency of hydrophobic air pollutants in the presence of ethanol may have resulted from improved sorption conditions. Cyclohexane is hardly soluble in water (up to about 55 mg dm^{-3} at 25°C), while it is easily soluble in ethanol (completely miscible)

(Yalkowsky et al., 2016). On the other hand, ethanol is easily absorbed in water, resulting in some content of ethanol in the mineral salt medium solution circulating in the biotrickling filter (discussed later, Fig. 3). Thus, the sorption efficiency of cyclohexane to mineral salt medium may be enhanced in the presence of ethanol. However, beside the fact that synergistic effects for simultaneous removal of hydrophilic and hydrophobic VOCs treated in biofiltration processes are already identified (Yang et al., 2018; Zhang et al., 2006), the actual mechanisms of this improvement are not yet defined.

Fig. 2A presents the performance of a biotrickling filter fed with other mode of feed gas supply: the treated gas stream contained cyclohexane and ethanol just from the process start-up. The following observations may be formulated when the course of a process performance (Fig. 2A) is compared with previously discussed courses (Figs. 1A and 1B). Firstly, the removal efficiencies of ethanol and cyclohexane are higher for both the start-up period (about two first weeks of biofiltration) as well as the steady-state conditions (after about 20 days of biofiltration) when two modes of feed supply are considered. Secondly, the removal performance with respect to cyclohexane is much higher in the second mode (Fig. 2A) than in the previous mode (Fig. 1B). Such observations may be explained by the increased metabolism of cyclohexane in the presence of ethanol, which resulted in promoted development of microbial flora within the biofilm and enhanced biofiltration performance with respect to cyclohexane. The following values of elimination capacity (EC) were reached for the investigated process performance: for cyclohexane, about 67.5 and 89 g m^{-3} h^{-1} (Fig. 1 and Fig. 2A, respectively) and about 35–36.7 g m^{-3} h^{-1} for ethanol. Biotrickling filtration of ethanol was studied by Cox at al. and Avalos Ramirez and co-workers, and the values of EC were reached as high as 220 and 970 g m^{-3} h^{-1}, respectively (Avalos Ramirez et al., 2007; Cox et al., 2001). For cyclohexane, elimination capacity of about 38 g m^{-3} h^{-1} was reached by Salamanca and co-workers for biotrickling filtration on polyurethane foam using *Acivodorax* sp. CHX100 (Salamanca et al., 2017). Please note that the mentioned studies dealt with the removal from air of single VOCs and the results presented in this paper reveal enhanced removal of cyclohexane in the presence of ethanol, allowing for much higher elimination capacity of cyclohexane than previously reported in the literature (up to 89 g m^{-3} h^{-1} in this study compared to about 38 g m^{-3} h^{-1} in reached by Salamanca and co-workers).

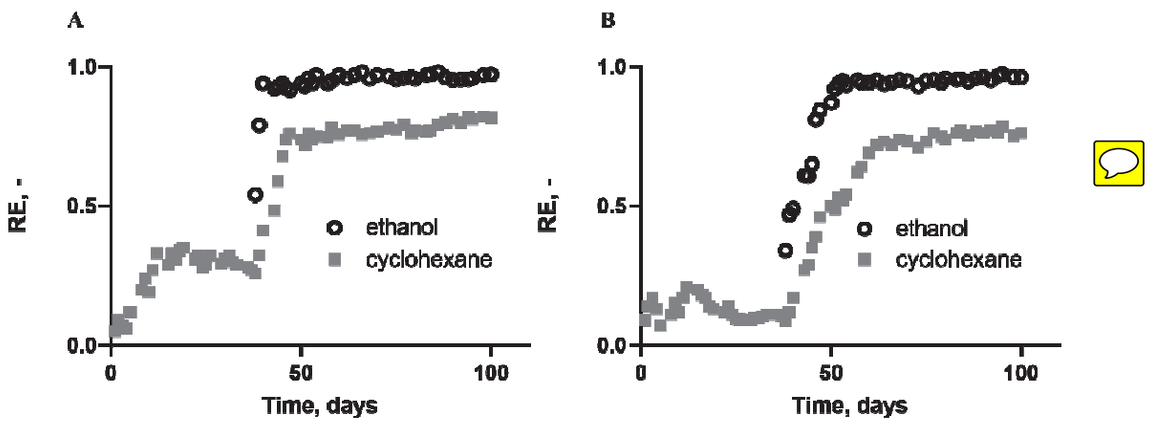

Fig. 1. Performance of a biotrickling filter inoculated with *Candida albicans* (Fig. 1A) and *Candida subhashii* (Fig. 1B). Inlet loading of cyclohexane: 45 g m^{-3} h^{-1} (C_{in} = 200 ppm v/v; days 1–20) and 90 g m^{-3} h^{-1} (C_{in} = 400 ppm v/v; days 21–100); inlet loading of ethanol: 36,9 g m^{-3} h^{-1} (ethanol introduced on 38th day of biofiltration; C_{in} = 400 ppm v/v).

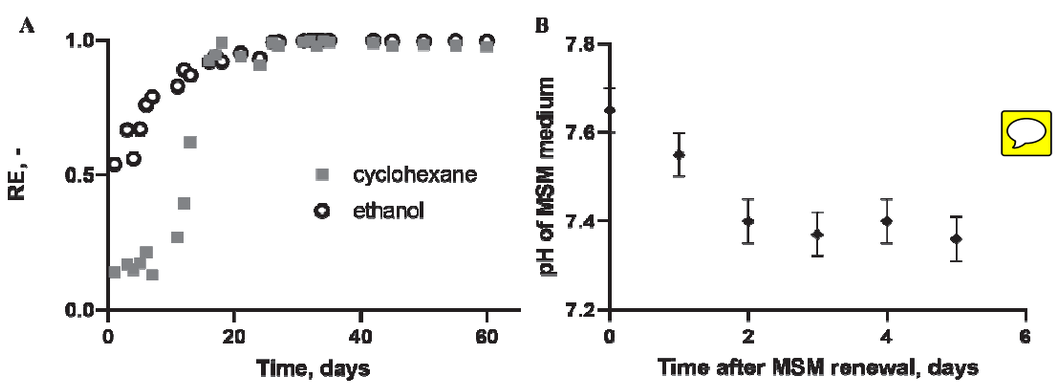

Fig. 2. Performance of a biotrickling filter inoculated with *Candida subhashii* (inlet loading for cyclohexane: 45 g m^{-3} h^{-1} (C_{in} = 200 ppm v/v; days 1–35) and 90 g m^{-3} h^{-1} (C_{in} = 400 ppm v/v; days 36–60); inlet loading for ethanol: 36,9 g m^{-3} h^{-1}; C_{in} = 400 ppm v/v) (Fig. 2A). Effect of biofiltration time on the pH of mineral salt medium (Fig. 2B).

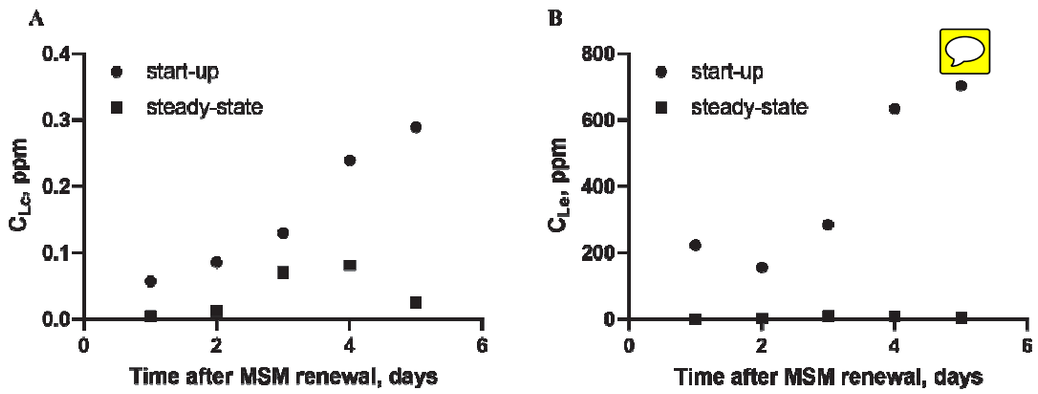

Fig. 3. Effects of biofiltration time on the composition of mineral salt medium with respect to cyclohexane (C_{Lc}, Fig. 3A) and ethanol (C_{Le}, Fig. 3B)

The effects of the gas stream composition on the physico--chemical parameters of the trickling liquid were investigated in order to evaluate possible effects on the process performance. Changes in pH of the trickling liquid as well as variations in the concentrations of treated compound in the liquid phase were evaluated. Fig. 2B shows changes of the pH values of MSM as a function of time after the introduction of a fresh portion of a trickling liquid (exchange of a trickling liquid was performed once a week throughout the whole time of experiments). The results show that only a slight pH drop of the solution was observed, probably due to products of microbial metabolism, typically leading to acidification of aqueous solutions, in the time period between the introduction of new portion of MSM and its exchange.

Interesting results are presented in Figs. 3A and 3B. The concentrations of the treated compounds were investigated in the trickling liquid for time intervals between the solution exchange, both for the start-up period as well as during the operation of biotrickling filters at steady-state conditions. During the start-up period, the concentrations of both cyclohexane and ethanol in the MSM solution increase with time after the solution renewal. It must be noted that due to the low solubility in water, the identified concentrations of cyclohexane are about 3 orders of magnitude lower than for hydrophilic ethanol. The increase of the target compounds concentrations may be explained by their partial absorption in the trickling liquid, which is especially true for ethanol. This is why the ethanol concentration in the MSM solution increases with time after a solution renewal during the start-up period (unsteady-state condition, Fig. 3B). As a result of absorption of ethanol vapors in MSM solution, its concentration in the gas phase decreases, thus a gas stream undergoing the biofiltration process is diluted. Therefore, the decrease of ethanol concentration in the outlet gas stream is attributed not only to the biofiltration process, but also partially to its absorption in MSM solution. Interestingly, the concentrations of ethanol in the MSM solution under steady-state conditions are very low, regardless of the time since the trickling liquid renewal. This observation suggests that a decrease of ethanol concentration in the outlet gas stream is predominantly the result of its biodegradation, i.e., the rate of biofiltration is high enough for its complete biotransformation and removal from the gaseous phase. Similar observations are valid for cyclohexane (Fig. 3A), bearing in mind its very limited solubility in aqueous solutions.

Optical microscopic analyses of fragments of biotrickling filter packing materials

Figures 4a to 4d present optical microscope images of biofilms formed on the structure of polyurethane foam applied as a packing material in the investigated biotrickling filters (Fig. 4e is a control showing polyurethane foam only). The below presented images were taken after the immobilization procedure (just prior to the introduction of inoculated packing elements to biotrickling filters) and after 100 days of biofiltration in order to evaluate the development of the biofilm. Optical microscopy observations reveal that both selected fungi species are able to form a biofilm over the biofilter packing elements and the thickness of developed biofilms is about 4 to 5 times higher than in the start-up period. Based on images presented in Figs. 4a to 4e it was found that the thickness of biofilm observed after the immobilization procedure was about 30 and 60 μm for *C. albicans* and *C. subhashii*, respectively, while the thickness of biofilm measured after 100 days of biofiltration was about 130 and 150 for μm for *C. albicans* and C. *subhashii*, respectively.

Flow cytometry analysis of applied fungi species

Cells of *Candida albicans* and *Candida subhashii*, sampled during the biofiltration start-up and after 100 days of biofiltration process, were subjected to flow cytometry analyses in order to determine the general condition of the fungi species. First series of analyses included a test with Annexin V which resulted in identification of apoptotic and necrotic cells. During the apoptosis, phosphatidylserine is transported from internal to external leaflet of the phospholipid bilayer. Annexin V is a specific reagent with high affinity to phosphatidylserine. In the commercial tests, Annexin V is conjugated with fluorescein. After binding with plasma membrane of apoptotic cells, green fluorescence may be detected. Additionally, propidium iodide is applied in this test, allowing for the differentiation of three main types of cells: alive, early apoptotic as well as necrotic and late apoptotic (Fig. 5a) (Chen et al., 2019; Henry et al., 2013; Vermes et al., 1995).

The results presented in Figs. 5b to 5e and in Table 1 show that the prominent fraction of cells remain alive after 100 days of biofiltration. Within a group of a given fungi species, the distribution of cell populations is similar for samples taken during the start-up period and after 100 days of biofiltration. This observation suggests that the same fungi species are

inhabiting the biotrickling filter packing elements as were those initially inoculated, indicating that no contamination of microbial cultures occurred.

The results presented in Table 1 inform that a decrease in the number of alive cells for both investigated fungi species is about 3%. It seems that the reduced number of alive cells results from a natural physiology of these microorganisms and it is not a result of the negative influence of the conditions induced by the biofiltration process on their viability.

Flow cytometry – evaluation of the cell cycle

Cytometric analysis of the cell cycle allow to determine the state of the cell population, i.e. how many of the cells in the analyzed population are actively dividing (i.e. are alive, denoted by index H-1 on the histogram), how many are at rest or how many die by apoptosis (denoted by index H-2 on the histogram). Membrane perforation, which is one of the stages of cell fixation during preparation for cytometric analysis, allows propidium iodide to enter the cytoplasm and the nucleus and, as a result, bind (intercalate) with double-stranded DNA (Martinez-Rojano et al., 2008; Ramani et al., 1997). The results presented in the attached histograms (Figs. 6a–6d) show that both *C. albicans* and *C. subhashii* remain in a good general condition, i.e., their cells stay alive, when biofilm samples taken during the process start-up and after 100 days of biofiltration are compared. During the start-up period, predominating cells remain in the interphase (H-1 = 96.5% for *C. albicans* and H-2 = 92% for *C. subhashii*), thus indicating that these cells are alive. After 100 days of the process, the number of alive cells slightly decreases at the expense of growth in the number of the dying cell population, indicated by H-2 index on the histograms. However, this is a natural process because the population is aging physiologically during the analyzed time interval and no drastic changes are observed that indicate

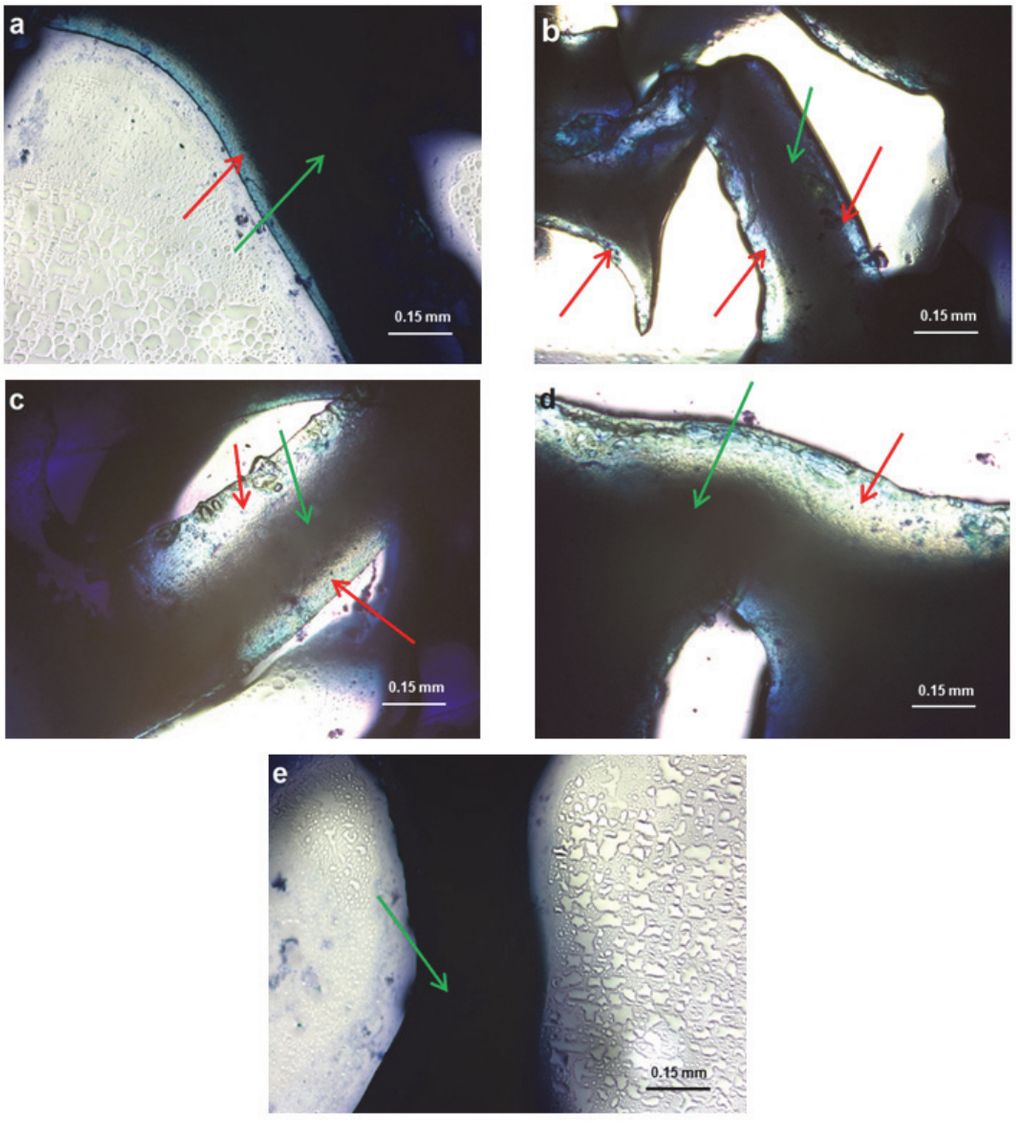

Fig. 4. Optical microscopy images (magnification: 10×; cells were stained with methylene blue): a – image of *C. albicans* biofilm after immobilization; b – image of *C. subshashii* biofilm after immobilization; c – image of *C. albicans* biofilms after 100 days of biofiltration; d – image of *C. subhashii* biofilm after 100 days of biofiltration; e – image of a fragment of polyurethane foam (control sample without a biofilm); red arrow – biofilm; green arrow – polyurethane foam

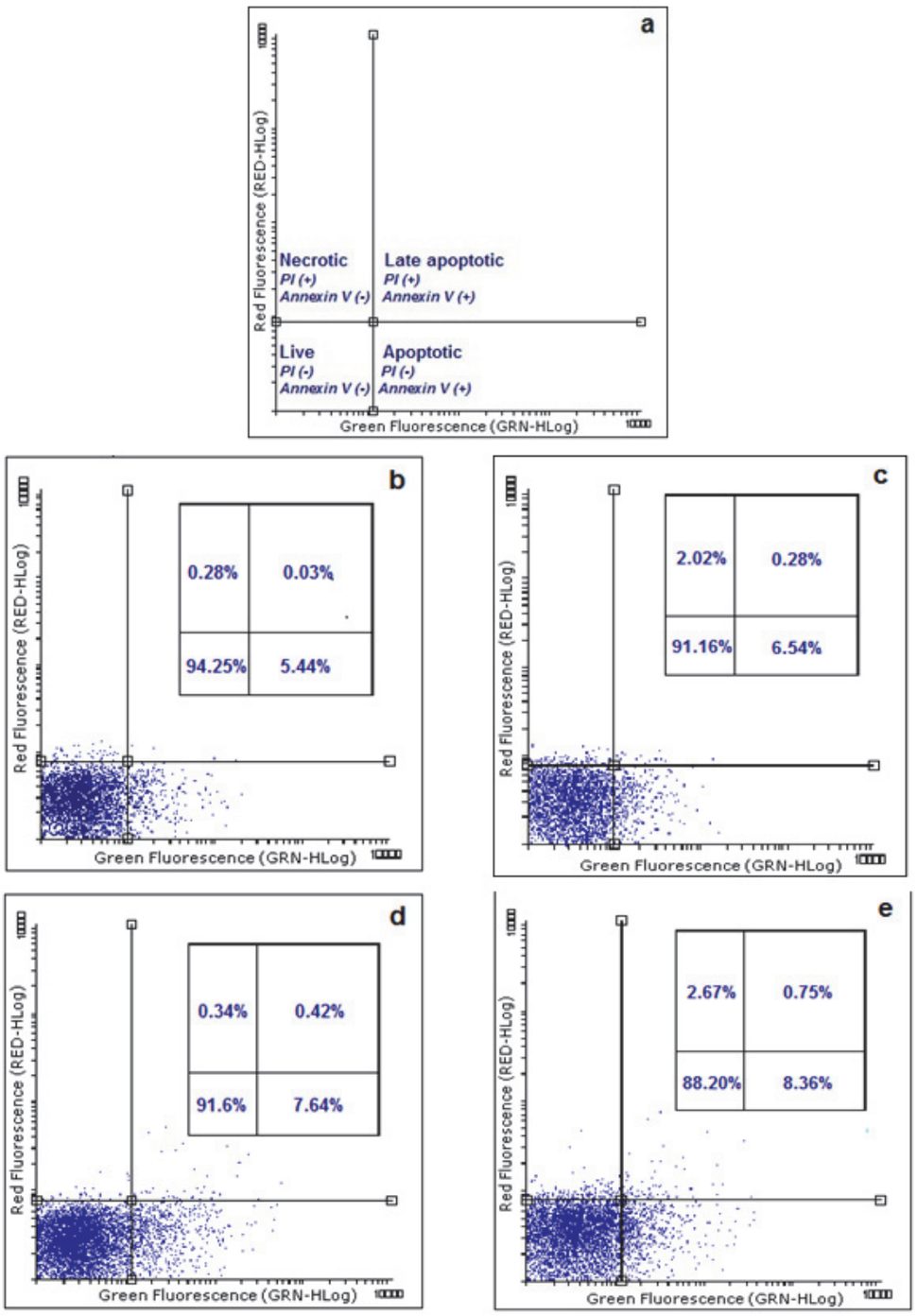

Fig. 5. Flow cytometry cytograms of cell viability: a – general information on how to read the cytogram; b – *C. albicans* after immobilization (start-up of the process); c – *C. subhashii* after immobilization (start-up of the process); d – *C. albicans* after 100 days of bilfiltration; e – *C. subhashii* after 100 days of biofiltration

Table 1. Summary of results obtained from Annexin V test

Species/Type of cell populations	Alive, %	Early apoptotic, %	Necrotic and late apoptotic, %
C. albicans (start-up)	94.25	5.44	0.31
C. albicans (after 100 days of biofiltration)	91.6	7.64	0.76
C. subhashii (start-up)	91.16	6.54	2.30
C. subhashii (after 100 days of biofiltration)	88.20	8.36	3.42

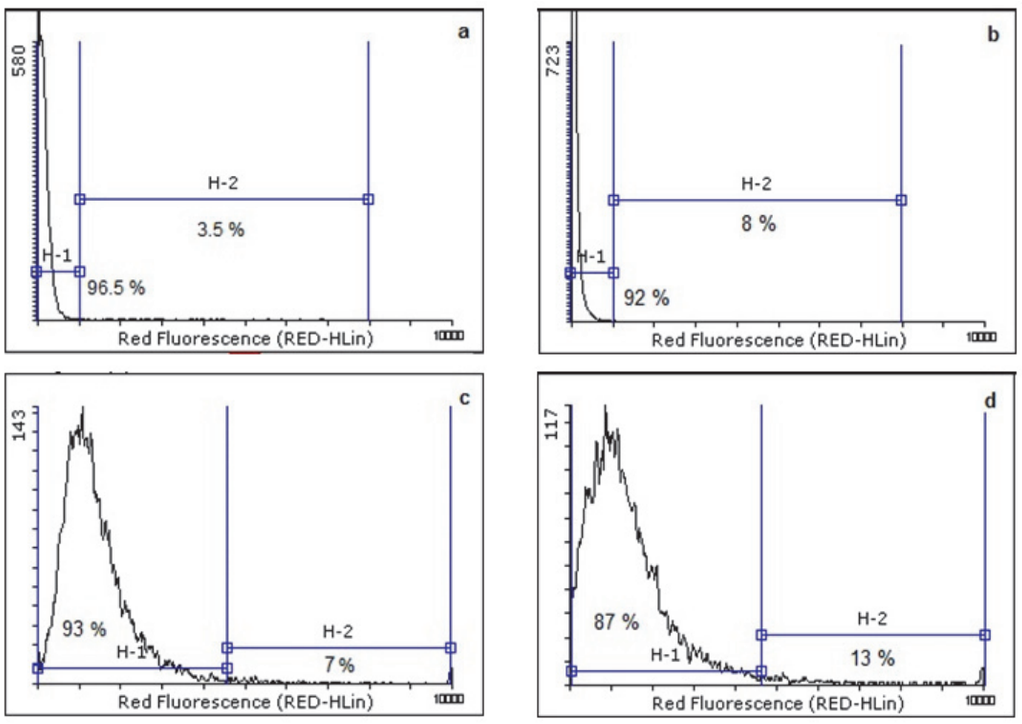

Fig. 6. Flow cytometry histograms of the cell cycle of investigated fungal isolates stained with propidium iodide: a – *C. albicans* after immobilization (start-up of the process); b – *C. subhashii* after immobilization (start-up of the process); c – *C. albicans* after 100 days of biofiltration; d – *C. subhashii* after 100 days of biofiltration

a negative impact of the process conditions on the viability of the species of investigated fungi. The results of flow cytometry analyses support the postulate that the use of *C. albicans* and *C. subhashii* in biofiltration processes can be upheld either as the main component of the inoculum or as additives that improve the process efficiency.

Conclusions

In this paper, *Candida albicans* and *Candida subhashii* species were inoculated to polyurethane-packed biotrickling filters to treat the air stream polluted with cyclohexane and ethanol. The obtained results of flow cytometry analyses indicate that the investigated fungi species may be applied to remove the above mentioned volatile organic compounds from their mixture with air. A stable biofilm has been formed on the packing elements of biotrickling filters, enabling a biofiltration performance with high removal efficiency. A synergistic effect of ethanol on the biofiltration of cyclohexane was identified. It was found that the removal efficiency of cyclohexane is higher when ethanol is introduced from the process start-up comparing to the case when it is introduced to a steady-state operating biotrickling filter, treating cyclohexane solely from the process start-up. Enhancement of biotrickling filtration of cyclohexane may result from both its co-metabolism with ethanol as well as improved sorption properties of trickling liquid for cyclohexane in the presence of ethanol, thus decreasing the mass transfer barrier for hydrophobic VOC. Additionally, investigations on the composition of liquid phase with respect to the treated compounds were performed. The results indicate that negligibly small concentrations of these compounds are found in the liquid phase in the steady-state conditions, when the removal efficiency of cyclohexane and ethanol are about 95–99%. The results of these studies indicate that the mechanism of enhanced removal of hydrophobic volatile organic compounds in the presence of hydrophilic compounds is rather complex and requires more in-depth elucidation, especially when multi-component systems are considered. In such future investigations, attention should be paid to link the gas-phase composition with changes occurring in the biofilm structure and composition as well as in the liquid phase, especially in the perspective of mathematical model formulation for biotrickling filtration of multi-component gas streams and specified single-component inoculum.

Acknowledgements

This research was funded by the Ministry of Science and Higher Education, Poland, through the specific subsidy awarded to Faculty of Chemistry, Gdańsk University of Technology (grant number 33882).

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Usuwanie z powietrza cykloheksanu i etanolu w biofiltrach strużkowych zasiedlonych grzybami Candida albicans i Candida subhashii

Streszczenie: W pracy przedstawiono badania nad usuwaniem cykloheksanu i etanolu z powietrza w boifiltrach zraszanych, wypełnionych pianką poliuretanową, zasiedloną grzybami z gatunku Candida albicans i Candida subhashii. Przedstawiono i omówiono wyniki dotyczące wydajności procesu (na podstawie pomiarów techniką chromatografii gazowej) wraz z wynikami cytometrii przepływowej dla utworzonego biofilmu. Uzyskano wartości zdolności usuwania, wynoszące około 89 g m^{-3} h^{-1} i 36.7 g m^{-3} h^{-1}, odpowiednio dla cykloheksanu i etanolu, gdy te związki jednocześnie poddawano procesowi biofiltracji w biofiltrze zaszczepionym Candida subhashii. Wyniki wskazują, że obecność etanolu powoduje zwiększenie skuteczności usuwania cykloheksanu z powietrza. Wzrost skuteczności usuwania z powietrza cykloheksanu w obecności etanolu może wynikać z polepszonego metabolizmu cykloheksanu w takich warunkach oraz z ograniczenia bariery dla przenikania masy, wskutek lepszych właściwości sorpcyjnych cieczy zraszającej wobec cykloheksanu w obecności etanolu.