Polish J. Environ. Stud. Vol. 15, No. 2 (2006), 335-340

Original Research

Trichloroethene Elimination from Air by Means of Biofoltration

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Received: February 22, 2005
Accepted: October 25, 2005

Abstract

The aim of this study was to develop a biofiltration system able to remove TCE from contaminated air and to optimize its operating conditions. Three types of one- and two-step installations were examined for the capacity to remove gas-phase trichloroethene from waste air. The immobilizate of the biomass active in TCE decomposition was used in the process. The efficiency of the process and metabolic activity of biomass were investigated.

The stable and effective biofiltration process was achieved when a two-step installation was used with hybrid bioreactor containing activated sludge suspension with active biomass immobilizate (bioscrubber) being the first step. The second step was a biofilter filled with traditional deciduous leaf debris compost mixed with wood chips and activated carbon. The efficiency of contaminant elimination achieved during 2.5 months of operation was between 52% and 92%. The bioscrubber activity in gas purification was high − even up to 76%, and biofilter capacity for contaminant elimination was in the range 65-88.3 g TCE/ m^3/h. Active strains of microorganisms selected in the course of the process were isolated and identified. Dominant bacteria belonged to the genus *Streptomyces*, *Sarcina, Arthrobacter*, *Bacillus* and also to Gram-negative rods from species *Agrobacterium radiobacter* and *Sphingomonas paucimobilis*. Moreover, two dominant strains of moulds and one strain of yeast were isolated from the biofilter-bed.

Keywords: waste gas, biofilter, bioscrubber, trichloroethylene

Introduction

Trichloroethene (TCE) is an unsaturated aliphatic chlorinated hydrocarbon widely used as a degreasing agent for metalworking, machine and electronic industries. TCE is known to be a carcinogenic substance, thus its loss to the ambient air may have an adverse impact on air quality and endanger public health. Therefore, it is very important to develop effective technologies for removing this harmful compound from the environment.

Physical and chemical methods of TCE removal such as combustion, adsorption and absorption are expensive and require elaborate equipment or fuel. These processes also generate secondary pollutants that require further treatment. Compared to these processes, biofiltration is a cost-effective method and degradation of volatile organic compounds (VOCs) results in non toxic substances such as carbon dioxide and water.

Contaminant elimination occurs by passing them through microbial layers formed in a filter-bed. This bed should be capable of adsorbing gaseous substances and supporting growth of microflora. The most commonly used packing materials are peat, compost, wood chips and soil. The filter materials could be either natural or synthetic and should have large surface area, high permeability and good sorption capacity. Other important

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properties of filter media are buffer capacity and moisture content [1].

Initially, biofiltration was mainly used to remove malodors and inorganic substances such as ammonia and hydrogen sulfide from air. Then, since the 1980s, its application has been extended to the removal of VOCs. The biodegradation of VOCs is affected by various environmental factors such as moisture content, pH, temperature, access to mineral nutrients and microelements. Other factors are the type of contaminant and VOC input rate. Contaminant bioavailability depends on physicochemical processes such as adsorption, desorption, diffusion and dissolubility. Maintenance of biofilter conditions suitable for microbial activity is essential for successful operation [2, 3].

Trichloroethene is a nonflammable, colorless liqud, highly volatile (boiling temp. 87^0C) and practically water insoluble (0.081 g/dm^3). Many researchers have reported that cometabolism was responsible for TCE degradation. Under aerobic conditions microorganisms utilizing a primary substrate such as methane, phenol or toluene and also ammonia oxidizers produce non-specific oxygenases that degrade TCE [3, 4]. The incomplete reductive dechlorination of trichloroethene was observed in a wide variety of methanogenic environments [5]. Experiments carried out in anaerobic conditions also revealed the possibility of TCE degradation to ethane by means of methanol-degrading bacteria [6].

Sukesan and Watwood [7] observed significant differences in TCE removal efficiencies between the two compost types in propane and methane-stimulated compost. Deciduous leaf debris compost removed more than 95% of 5-50 ppm TCE concentration in gas stream, whereas less than 15% removal was observed under similar conditions with wood chips and bark compost. Hecht et al. [8] demonstrated that in the presence of phenol as a co-substrate the removal efficiency of TCE in a column bioreactor varied between 30--80% and was limited by the biodegradation rate.

In this work we studied the effects of operational conditions such as contaminant load and the air volumetric load on the removal of TCE. After 251 days of operation the optimal operating conditions were determined.

The objective of the study was to isolate and characterize an appropriate microbial culture for trichloroethene degradation, to select of proper biofilter matrix content and to optimize operating conditions for the biofiltration system.

Materials and Methods

Microorganisms

Isolation of Bacterial and Fungal Strains Capable of Using TCE as a Sole Source of Carbon and Energy

Microbial acclimation to TCE was performed by sequential subculturing on mineral medium with TCE and yeast extract using activated sludge as the first inoculum. Simultaneously, the Sequence Batch Reactor system containing activated sludge was employed.

Batch cultures were performed in Erlenmayer flasks on a rotary shaker with a basal mineral medium containing (per dm^3): $(NH_4)_2SO_4$ - 0.50 g, KH_2PO_4 - 1.36 g, Na_2HPO_4 - 2.25 g, $FeSO_4$ - 0.01 g , $MgSO_4$ - 0.2 g, $Ca(NO_3)_2$ - 0.05 g, trace elements solution - 0,1 cm^3, vitamins solution - 1 cm^3. As a source of carbon and energy for microorganisms, TCE and yeast extract were applied. TCE emulsion in water was obtained after treatment by ultrasonic waves. Primary cultures were inoculated with the activated sludge (100 cm^3 of the culture inoculated with 5 cm^3 of activated sludge). After 48 h of incubation they served as material for the inoculation of new series. In succeeding subcultures the concentration of yeast extract was diminished and the content of TCE was increased. In total, six subcultures were performed. In primary cultures yeast extract and TCE concentrations were 1000 mg/dm^3 and 100 mg/dm^3, respectively. Finally, cultures were performed on medium with TCE as a sole source of carbon and energy in concentrations of 1000 mg/dm^3.

Selection of bacteria and fungi active in TCE degradation was carried out parallel in the same media, the only difference being pH - 6.5 for bacteria and 5.0 for fungi. Selected strains were isolated on mineral medium agar plates supplemented with TCE in concentrations of 770 mg/dm^3.

Activated sludge culture was performed in the Sequence Batch Reactor working in four 6-hour cycles per day. The reactor was fed with synthetic domestic wastewater [9] supplemented with TCE. Trichloroethene was applied dissolved in ethanol. The loading was doubled every week. After one month of working, microbial strains active in TCE degradation were isolated on solid medium, as described previously. Isolated strains were applied to biofilter as an immobilizate in wood chips. Immobilization carrier was prepared before use by keeping in alkaline solution (pH 9-10), rinsing with distilled water and drying at 105^0C. Bacterial preculture for immobilization medium inoculation (100 ml in Erlenmeyer flask) was incubated in mineral medium supplemented with peptone (0.1%) and TCE (0.1%) on a rotary shaker for two days at 26^0C and 120 rpm. Immobilization culture was performed on a magnetic stirrer in 5 dm^3 vessels with the same medium. After 24 h of incubation dry wood chips were added (medium to wood chips ratio was 2:1 v/v). Adsorption was carried out in the next 24 h in aerating conditions. Wood chips, after separation from the medium, were added to the biofilter-bed.

Experimental System

The biofilter column was made of plexiglas and has the following dimensions: ID-0.2 m, length 1.2 m. It was packed with compost mixed with wood chips (2:1 v/v), being the carrier for the biomass active in contaminant degradation. The height of the biofilter-bed was 1 m and volume - 0.0314 m^3. At the final stage of the process the

bed was supplied with activated carbon containing immobilized biomass active in TCE degradation (the volumetric ratio of the filter-bed matrix to the activated carbon was 14:1).

Figures 1 and 2 show schematic diagrams of the biofilter systems employed at the first, second and third stages of the experiment.

Pressurized air coming into the system was divided into two streams. During the first stage of the experiment one stream was passing the TCE reservoir, the other was going through the tank with distilled water. Both streams were joined in the mixing tank and after passing the flow-meter, were going upstream through the biofilter. During the second and third stage of the process two steps of the contaminated air treatment were employed. As shown in Fig. 2, contaminated air was passing the bioscrubber containing activated sludge suspension mixed together with immobilizate of active biomass. Then the air, after passing the mixing tank, was flowing to the biofilter through the flow-meter. The humidity of air coming to the biofilter was 100%.

Operational parameters such as the incoming TCE concentration, air flow velocity and the pressure drop varied in the course of the process. Moisture content of the filter-bed was held to 60-80% WHC_{max} (maximum water holding capacity). It was determined by changes in weight after drying the bed material for 12 h at 105^0C. In the course of the first and second stage of the experiment the filter-bed was periodically sprayed with active biomass suspension in order to assure proper microbial and moisture content.

Sampling Procedure

Filter-bed material was taken by the sampling ports situated 25 cm and 75 cm from the bottom; samples were described as the "lower layer" and "higher layer", respectively. Air samples for gas chromatography analysis in one-step installation were taken from two sampling points: directly under the biofilter-bed and 10 cm up from the filter-bed surface (Fig. 1). Air samples for gas chromatography analysis in two-step installation were taken from three sampling points: the valve installed before the bioscrubber (inlet contaminated air), directly under the biofilter-bed (air after first stage of treatment) and 10 cm up from the filter-bed surface (outlet purified air) (Fig. 2).

Analytical Procedure of TCE Estimation

Samples of contaminated and purified air were taken by the sampling points. The air was passed through a glass tube filled with 100 g of active carbon with flow rate 30 dm^3/h for 10minutes (contaminated air) and 30 minutes (purified air). Collected contamination was desorbed from carbon using 1 ml CS_2. A sample of 1 µl prepared solution was manually introduced into the GC injection port.

Since day 142 of the experiment, samples of 10 µl contaminated air were introduced into the GC by manual injection using a gas-tight syringe.

Analysis of trichloroethene in the contaminated air was performed with a Hewlett Packard gas chromatograph GC 5890A (Hewlett Packard, USA) equipped with a HP-5 Crosslinked 5% PH ME Siloxane column (30 m x 0,53 mm x 1,5 µm; HP Part No. 19095J-323, USA). The detector was a flame ionization detector (FID) operated at 160^0C. Injector temperature was 160^0C. Split ratio 1:10 was used. The analysis was carried out isothermally at 80^0C. Helium was used as a carrier gas. The flow rate of He to the column was 5.2 cm^3/min. The responses from FID were collected and analyzed by computer.

Microbiological and Enzymatic Activity Estimation

Number of bacteria was determined by plating on nutrient agar (for heterotrophic count) and on mineral medium with TCE as a sole source of carbon. Biofilter bed suspensions were prepared by shaking 10 g of material with 90 cm^3 of sterile sodium pyrophosphate (0.1% solution) for 30 min at 20^0C and 120 rpm. 0.1 cm^3 of appropriate dilutions were surface spread onto agar plates. Incubation temperature was 26^0C. Colony-form-

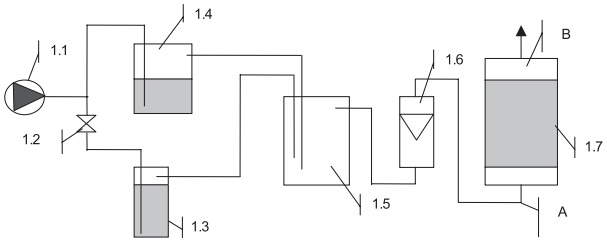

Fig. 1. Schematic diagram of the biofilter system employed at the first stage of the experiment: 1.1 - membrane pump for air supply, 1.2 - valve,1.3 - TCE reservoir, 1.4 - water container, 1.5 - mixing tank, 1.6 - gas flow meter, 1.7 - biofilter, A, B - sampling points.

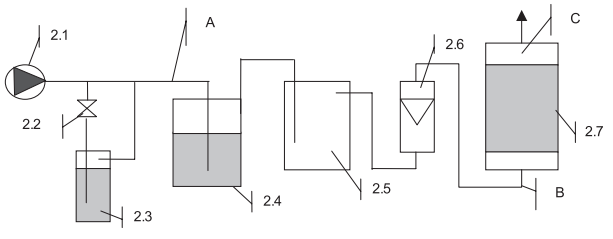

Fig. 2. Schematic diagram of the biofilter system employed at the second and third stage of the experiment: 2.1 - membrane pump for air supply, 2.2 - valve, 2.3 - TCE reservoir, 2.4 - bioscrubber, 2.5 - mixing tank, 2.6 - gas flow meter, 2.7 - biofilter, A, B, C - sampling points.

ing units for total heterotrophic bacteria were counted after 2 days, TCE-degrading colonies were determined after 7 days.

Taxonomic estimations of bacterial strains active in contaminant decomposition were accomplished by means of standard identification test API 20 NE (Bio-Merieux). The final identification was performed according to "Bergey's Manual of Determinative Bacteriology" [10].

The activity of dehydrogenases was assayed by measuring triphenyl formazan (TF) formation - the product triphenyl tetrazolium chloride (TTC) reduction at the respiration chain pathway. The modified method was elaborated according to [11]. Samples were prepared by shaking 10 g of biofilter matrix in 90 cm^3 of distilled water. Aliquots of 5 cm^3 were taken to analysis. All reagents were added according to Polish standard [10]. Samples were incubated for 24 h in a rotary shaker (120 rpm) at 26^0C. Further analytic procedure was performed according to [12]. The TF concentration was determined in a spectrophotometer at 490 nm wavelength. Specific enzyme activity was given in µmol TF per kg protein per second.

Protein concentration was assayed using Lowry's method. Protein was determined in a spectrophotometer (at 750 nm) in cell-free extracts obtained after ultrasonic disintegration.

Hydrolase activity was measured by means of modified fluorescein diacetate (FDA) assay, according to Schnürer and Rosswall [12]. Hydrolytic degradation of FDA was performed in shaking cultures (120 rpm, 26^0C) containing 10 g of biofilter matrix, 50 cm^3 of phosphate buffer (pH 7.6) and fluorescein diacetate in concentration 20 µg/cm^3. After 24 h the reaction was stopped by adding acetone in volumetric ratio 1:1. Fluorescein concentration was assayed in spectrophotometer at 490 nm. Specific enzyme activity was given as mg fluorescein per kg protein per second.

Determination of the Organic Substance Content at the Filter Matrix [13]

Samples of filter bed material were dried at 105^0C and then incinerated at 550^0C. The organic substance content was determined by the weight difference between the dried and the incinerated samples.

Results

First and Second Stage of the Process

Experiments were carried out at the following operational parameters (Fig. 3, 4):

- contaminated air flow rate - 0.5-1.1 m^3/h
- the range of TCE concentration in inlet air - 1-37 g/m^3
- gas volumetric loads range from 16 to 35 $m^3/m^3/h$
- the range of contaminant loads coming to biofilter - 11.9-1309.7 $g/m^3/h$

For the second stage of the process, after bioscrubber installation:

- gas volumetric loads range for bioscrubber - 83-200 $m^3/m^3/h$
- contaminant loads coming to bioscrubber - 250.9-6581.6 $g/m^3/h$

The efficiency of contaminant elimination was between 1% and 83%; it was characteristic that neither loads of the filter-bed nor reinoculations influenced removal efficiency of TCE (Fig. 4). Stabilization of the process was not achieved.

The bacterial count in filter-bed varied from 0.5 to $3.0*10^8$ CFU/g dry matrix, the number of fungi was between 0.3 to $2.5*10^5$ CFU/g dry matrix. The content of microorganisms was higher in the "higher layer" than in the "lower layer" of the filter-bed. It should be pointed out that the number of microorganisms active in contaminant degradation increased very quickly and sometimes was even equal to the total number of microbes (Figs. 5, 6). Enzymatic control of the filter-bed biomass revealed an intensive increase in activity of hydrolytic enzymes and dehydrogenases within the first two weeks of the process (Figs. 7, 8, 9). The hydrolytic activity was at the range of 0.7-2.5 mg fluorescein/kg protein/s and dehydrogenases activity was between 0.4-1.5 µmol TF/kg protein/s for respiration without exogenous source of carbon and 0.2-

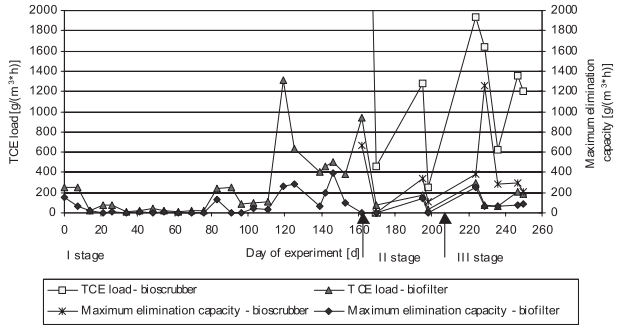

Fig. 3. The biofilter operation - TCE load and the maximum elimination capacity.

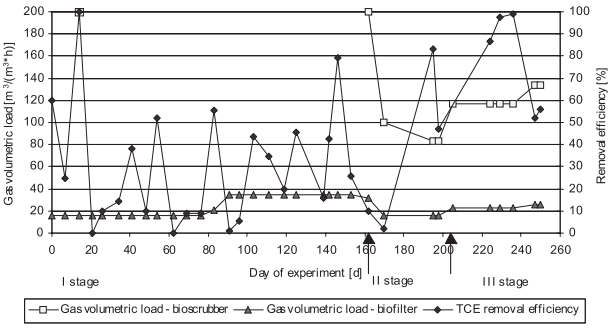

Fig. 4. The biofilter operation - gas load and removal efficiency.

4.4 µmol TF/kg protein/s for glucose containing samples. Activity values were slightly higher for the "lower layer" samples than for "higher layer."

The Third Stage of the Process

At this stage of the process an important change in content of the biofilter-bed was done. A small amount of activated Norit SX 2 carbon containing immobilized active biomass was mixed together with former biofilter matrix.

Operational parameters were as follows (Fig. 3 and 4):

- contaminated air flow rate - 0.7-0.8 m^3/h
- range of TCE concentration in inlet air - 5.3-16.5 g/m^3
- gas volumetric loads range - from 22.3 to 25.5 $m^3/m^3/h$
- contaminant loads coming to biofilter - 65.6-296.2 g m^3/h
- gas volumetric loads range for the bioscrubber - 117-133 $m^3/m^3/h$
- contaminant loads coming to bioscrubber - 625-1933 $g/m^3/h$

Biomass and water content was not supplied.

The efficiency of contaminant elimination achieved during 2.5 months of operation was between 52% and 92%. It should be pointed out that in the first days of this stage of operation the efficiency was very high - at the range of 86.5%-99%. At this time the bioscrubber was very effective in contaminant load elimination - even up to 76%. At the final part of the process bioscrubber effectiveness diminished - TCE load elimination in the first step of treatment was only up to 21% (Fig. 4). Presumably, the reason of this phenomenon was too long a time for bioscrubber exploitation.

At this stage the stabilization of the biofiltration process was achieved - the biofilter capacity for contaminant elimination was 65-88.3 g TCE/m^3/h (Fig. 3).

The microbial count in the filter-bed did not remarkably change comparing first and second stages of the process (Figs. 5, 6). Hydrolytic enzyme activity also was at the same level (Fig. 7). However, results of dehydrogenases estimations showed that this activity was strongly enhanced. In glucose-containing samples up to a 10-fold increase was observed compared to previous results (Fig. 9).

It should be pointed out that contaminant elimination was the result of biodegradation. Estimations of the organic substance content in the biofilter bed revealed that it had been stable during the process and kept at a level of 0.3-0.4 g/g dry matrix.

The only disadvantage connected with activated carbon application was a significant pressure drop that occurred at the biofilter - even up to 3,400 Pa/m. This phenomenon causes some difficulties in the operation of the air decontamination process.

At the third stage of the process the isolation and identification of the active strains of microorganisms was done. Dominant bacteria belonged to the genus *Sarcina, Arthrobacter*, *Bacillus* and also to Gram-negative rods from the species *Agrobacterium radiobacter* and *Sphingomonas paucimobilis*. Bacteria from genus *Streptomyces* played an important role at the process - numbers of these microorganisms were about 36% within the total number of bacteria active in TCE degradation. Moreover, two dominant strains of moulds and one strain of yeasts were isolated from the biofilter-bed.

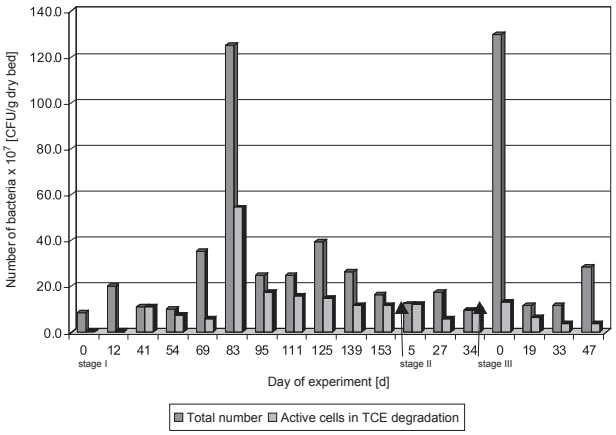

Fig. 5. Bacterial content in biofilter bed.

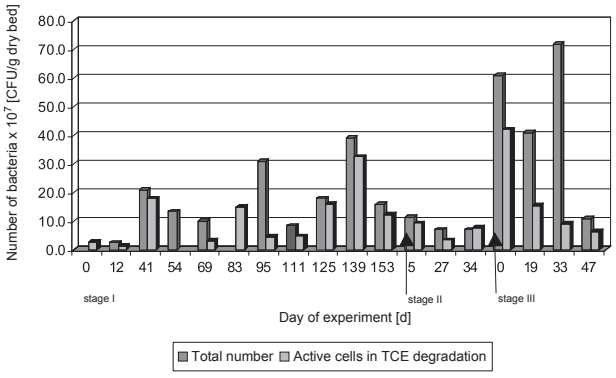

Fig. 6. The content of fungi in biofilter bed.

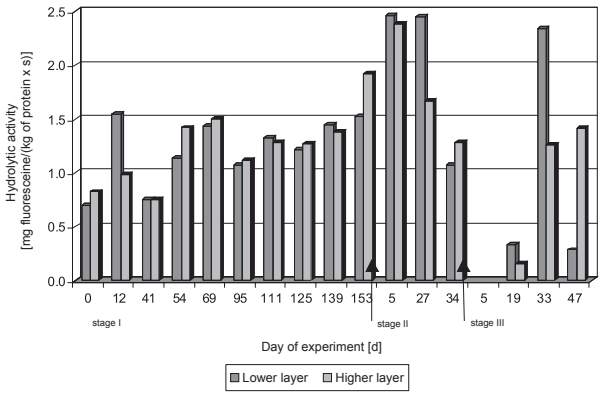

Fig. 7. Hydrolytic activity of biofilter microflora.

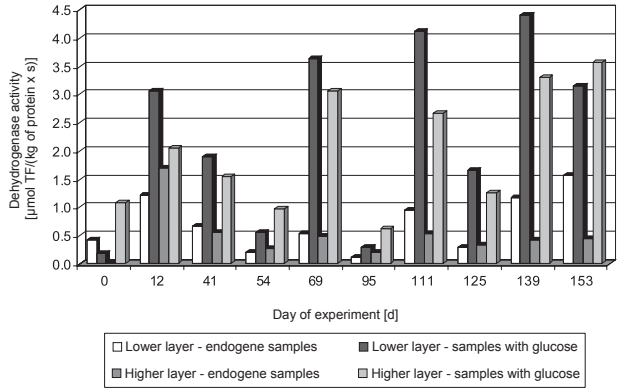

Fig. 8. Dehydrogenase activity of biofilter microflora during the first stage of the experiment.

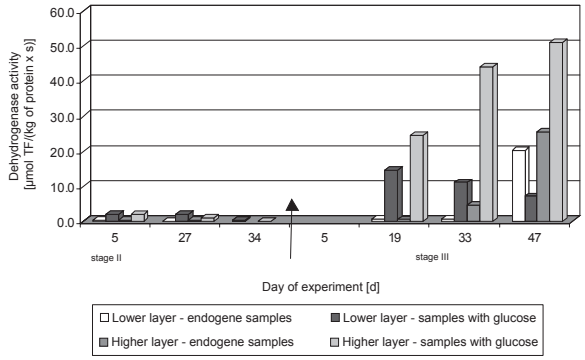

Fig. 9. Dehydrogenase activity of biofilter microflora during the second and third stage of the experiment.

Conclusions

The following conclusions are based on the findings of this study:

1. Biological decomposition of trichloroethene is possible in aerobic conditions while this compound is the only contaminant in the air.
2. Extremely poor water solubility and low boiling temperature of TCE make air purification possible only by passing it through a biofilter packed with compost supplied with active microorganisms immobilized in wood chips.
3. The bioscrubber addition to the system and the application of activated carbon to the filter-bed enables effective sorption of TCE and then its elimination by biodegradation.
4. Experiments allowed establishment of the contaminant removal rate - it was 65-88.3 g TCE/m^3/h.
5. Optimal conditions of TCE elimination process resulted in significant enhancing of the respirating activity of the biofilter bed biomass.
6. Activated carbon addition caused significant pressure drop at the installation; this phenomenon results in the increase of the technology costs.
7. During the subsequent process, microorganisms were selected from the filter-bed: Gram-negative rods from species *Agrobacterium radiobacter* and *Sphingomonas paucimobilis,* bacteria from genus *Sarcina, Arthrobacter* and *Bacillus*. Additionally, dominants from genus *Streptomyces*, moulds and yeasts.

References

1. YOON IN-KIL, PARK CHANG-HO. Effects of gas flow rate, inlet concentration and temperature on biofiltration of volatile organic compounds in peat-packed biofilter. Journal of Bioscience and Bioengineering **93**, 165, **2002**
2. KIM J.O., TERKONDA P.K., LEE S.D. Gaseous CAH removal by biofiltration in presence and absence of a nonionic surfactant. Bioprocess Engineering **19**, 253, **1998**
3. LEE S., MOE W.M., VALSARAJ K.T., PARDUE J.H. Effect of sorption and desorption resistance on aerobic trichloroethene biodegradation in soils. Environmental Toxicology and Chemistry **21**, 1609, **2002**
4. ARP D.J., YEAGER C.M., HYMAN M.R. Molecular and cellular fundamentals of aerobic cometabolism of trichloroethene. Biodegradation **12**, 81, **2001**
5. DISTEFANO T.D., GOSSETT J.M., ZINDER S.H. Reductive dechlorination of high concentrations of trichloroethene to ethane by an anaerobic enrichment culture in the absence of methanogenesis. Applied and Environmental Microbiology **57**, 2287, **1991**
6. PARVATIYAR M.G., GOVIND R.,BISHOP D.F. Treatment of trichloroethene (TCE) in a membrane biofilter. Biotechnology and Bioengineering **50**, 57, **1996**
7. SUKESAN S., WATWOOD M. E. Continous vapor-phase trichloroethene biofiltration using hydrocarbon-enriched compost as a filtration matrix. Applied Microbiology and Biotechnology **48**, 671, **1997**
8. HECHT V., BREBBERMANN D., BREMER P., DECKWER W.-D. Cometabolic degradation of trichloroethene in a bubble column bioscrubber. Biotechnology and Bioengineering **47**, 461, **1995**
9. PN-87 C-04616.10 Water and wastewater. Special test for sediments. The cultivation of standardized activated sludge in laboratory conditions.
10. HOLT J.G., KRIEG N.R. (eds). Bergey's manual of systematic bacteriology. Williams and Wilkins, Baltimore, Hong-Kong, London, Sidney, **1985-1989**
11. PN-82 C-04616.08. Water and waste water. Special tests for sediments. Determination of dehydrogenase activity in the activated sludge by spectrophotometric method with TTC.
12. SCHNÜRER J., ROSSWALL J. Fluorescein diacetate as a measure of total microbial activity in soil and litter. Appl. Environ. Microbiol. **43**, 1256, **1982**
13. PN-88 B-04481. Building soils. Laboratory tests.