QUANTITATIVE MICROBIOLOGICAL INVESTIGATION OF ACTIVATED FOAM
AND ZEOLITE CHARGES USED FOR BIOLOGICAL AIR TREATMENT

Pranas Baltrenas, Alvydas Zagorskis

Vilnius Gediminas Technical University, Department of Environment Protection
Saulėtekio al. 11, LT-10223, Vilnius-40, Lithuania
E-mail: pbalt@ap.vgtu.lt
alvydas@ap.vgtu.lt

Abstract. In recent years complex treatment technologies including not only microbiological but also other
efficient air treatment methods have been increasing applied. The highest effect is achieved when different
methods are optimally combined together. With the aim to improve the efficiency of volatile organic compounds
removal from the air, the biological and adsorption air treatment methods are combined in the work. The
investigation showed that microorganisms participating in the process of biological treatment could also
reproduce in the charges of inorganic origin composed of natural zeolite and foam. When these charges are
mixed with wood chips the microbiological activity of the medium is increased. A charge composed of the
mixture of wood chips and zeolite, 150 mm thick, was distinguished by the best treatment qualities. The highest
microbiological activity in this charge was achieved when air contaminated with butanol was passed through it at
the temperature of 30 °C. The number of units forming the colonies of microorganisms in zeolite amounted to
19.8 x 10⁴ cfu/g, and the concentration of butanol decreased from 303 mg/m³ to 219 mg/m³. A high level of
contaminant removal will be achieved upon increasing a filtering layer.

Keywords: biofiltration, sorption, destructors, microorganisms.

1. Introduction

One of the main issues important to many
countries of the world is rational use of natural
resources and protection of the environment from
different contaminants that are harmful not only to the
environment but to human health as well [1].

Branches of industry, such as chemical, varnishes
and paints production, oil refinement or food
industries, use a lot of organic substances, which in
different ways access the atmosphere. The most
widely spread organic compounds include acetone,
butanol, toluene, xylene and others [1, 2, 3].

Presently, one of the most promising air
treatment methods is biological air treatment using
certain cultures of microorganisms. The application
of this method is prospective when spontaneous
cultures of microorganisms are cultivated in a charge.
In this case biological air treatment is cheap, efficient
and does not produce secondary contaminants [1].

The capacity to use organic compounds is typical
of many representatives of microorganisms groups
but not of certain forms of narrow specialisation.
Microorganisms oxidising hydrocarbons is an
important group of organisms participating in the
cycle of carbon metabolism. The most important in
this group are bacteria and micromycetes together
with bacteria forming the largest part of the group.
Bacteria are capable of taking different hydrocarbons
from the environment and characteristic of a short life
cycle. The most widely spread genera such as
Arthrobacter, Acinetobacter, Pseudomonas, Bacillus,
Flavobacterium, Mycobacterium, Micrococcus,
Rhodococcus, have bacteria capable of oxidising
hydrocarbons. Microorganisms from over 70 genera
decompose hydrocarbons [4, 5, 6].

A number of species decomposing hydrocarbons
were identified among micromycetes as well. The
most frequently discovered species of micromycetes
are of the Penicillium, Aspergillus, Cladosporium,
Alternaria, Botrytis, Fusarium, Mucor genera [7].

The biodegradation intensity of aromatic
compounds depends on the number of rings and
condensation degree of a structure. The higher is the
level of condensation of a multi-ring compound the
slower is its mineralization. It is stated that the
capacity of microorganisms to decompose aromatic
compounds is nearly inversely proportional to the
number of rings in their structure. The decomposition
of separate hydrocarbons having four and more rings and a condensed structure takes place slowly.

The most important factor determining the speed of microbial reproduction and intensity of biochemical reactions is temperature. Different groups of microorganisms are adapted for living in different temperature. Like in case of other organisms, temperature for microorganisms can also be the minimum, optimum or maximum one. When temperature falls below the minimum or rises above the maximum one vitally important processes are disrupted. The reproduction of microorganisms is the fastest at the optimum temperature. For instance, the genera of psychrophilic and mesophilic microbes, such as *Pseudomonas* and *Achromobacter*, can reproduce in the temperature range of 10 to 30 °C. To improve the treatment efficiency of a biofilter, the optimum temperature of a biomedium has to be maintained in a device.

*Pseudomonas* culture is the most widely spread in nature. It actively participates in the destruction processes of organic compounds. The species of *Pseudomonas fluorescens* bacteria is found in water and on plants’ surface. The bacteria *Pseudomonas fluorescens* of the *Pseudomonas* genus evolved from water and wood substrates accounted for 55 and 67 %, respectively [8].

The main element of a biological air treatment device is a filtering medium, necessary as a substrate of microorganisms and at the same time supplying them with required nutrients. The following charges of natural origin are used in practice as the filtering media: compost, peat, wood chips, bark and activated sludge [9, 10].

With the aim to prolong the useful life of charges, artificial charges composed of polyurethane, propylene, polyethylene, glass, ceramic balls and other materials are also used. All these materials are destructed by microorganisms in the course of time [1, 11, 12].

In order to extend the useful life of a charge and at the same time increase the treatment efficiency of a device, several treatment methods, biological and adsorption, can be combined. When wood chips are mixed with zeolite, the charge’s life is extended and the sorption properties of the filtering medium are improved [13]. The cultures of spontaneous microorganisms will be able to develop not only in wood chips but also in inorganic zeolite [14]. Microorganisms that accumulate in a biofilm formed on the zeolite surface will decompose organic compounds accumulated in zeolite’s pores. To achieve better sorption properties of the charge, chips can be mixed with charges having a larger sorption surface, such as foam. In this case the charge will be distinguished by better properties of humidity sorption, low density, cheapness and a big area of treated surface.

The aim of investigation is to measure the number of microorganisms in the samples of foam and zeolite depending on the type of a supplied contaminant and air temperature maintained in the biofilter.

2. The methods of investigation

Experimental tests are performed using a biological air treatment device – a biofilter. The main element of the biofilter is a filtering activated charge. The charge used for tests was obtained by mixing zeolite grains (grain fraction: 10 – 12 mm) and foam cubes (cube dimensions: 30 x 30 x 20 mm) with wood chips. In this way, attempts are made to increase the biological activity of microorganisms in charges.

According to volume, zeolite granules were mixed with wood chips at the ratio of 50 x 50 %. The biofilter’s cassette, the second one from the bottom, was loaded with this charge. The third cassette of the biofilter was loaded with the charge of foam cubes and wood chips mixed at the same ratio.

Each layer of the charge is 0.5 m long, 0.48 m wide and 0.15 m high. After filling up the drawers the charge is activated.

![Fig 1. Laboratory biofilter’s stand (a); biofilter cassette’s section (b): 1 – tubular electric heaters, 2 – sieve, 3 – water sprayers, 4 – charge separation meshes, 5 – measuring places; cassette loaded with a charge (c)](image)

Prior to setting the biofilter for operation, the charge is moistened with water sprayers installed above every layer. In order to maintain a uniform airflow and make excess water flow to an excess water reservoir installed in the bottom part of the filter, the layers of the biomedium are separated from each other by metal meshes. Humidity in the charge is controlled by a weighted method [15]. To maintain the humidity of the entire charge volume (0.0675 m³) around 5 l of water are sprayed on the charges per day. Sampling points of the charge used to measure humidity are shown in Figure 2.

To ensure the growth of energy of microorganisms, a solution of mineral salts supplying microorganisms with vitally important biogenic elements is necessary. The solution of salts consists of: K₂HPO₄ – 1g, KCl – 0.5g, MgSO₄7H₂O – 0.5g,
FeSO₄·7H₂O – 0.1g, NaNO₃ – 0.90g, water – 1 000g. This solution is poured into water reservoir and sprayed over each layer of the charge. In order to have a spontaneous existence of microorganisms, the required acidity of pH=7.0 has to be maintained in the biomedium. Buffer solutions of sodium and potassium hydroxides are used to maintain the acidity. Biomedium’s acidity is measured with pH-meter [16, 17].

Air heaters installed by filter walls are used to maintain the required temperature of the biomedium. During charge activation these heaters help maintain the steady air temperature of 30 °C in the biofilter.

Different concentrations of acetone are fed to maintain the energy of microorganisms. Microorganisms use acetone as food and emit the products of metabolism, CO₂ and water, to the environment. Different concentrations are obtained by heating contaminants on an electric stove. The temperature of supplied vapour varies in the range of 20-50 °C. The initial concentration of supplied acetone was 23 mg/m³. The contaminant was supplied to the device 4 times a day for the period of 15 min. every time. Later, the concentration of the organic compound was increased every 2 days by 20 mg/m³ and the duration of acetone supply was extended to 1 hour. The charge was activated for 2 weeks.

To measure the number of microorganisms, control samples of zeolite and foam were taken prior to charge activation. The samples were taken in different places of charge’s cross-section according to the mesh principle.

**Fig 2.** Sampling points in the samples of zeolite (a) and foam (b)

After activating the charge, the concentration of acetone was periodically supplied for 3 days. The steady temperature of 20 °C was maintained with the help of tubular electric heaters. The velocity of airflow passed through the filter was 0.15 m/s. To maintain a uniform airflow velocity, an airflow control valve was installed in the biofilter.

Prior to sampling, airflow velocity and temperature were measured with the German meter Testo 452.

To evaluate the number of bacteria and micromycetes, the samples of zeolite and foam were taken. Sampling places are shown in Fig 2. The samples were taken with tweezers and placed into pre-heated weighing bottles by indicating the contaminant’s name, date and maintained temperature. These weighing bottles were placed into a hermetic wooden box and transported for microbiological testing.

Upon sampling completion, airflow temperature in the filter was increased to 25 °C with a thermostat. Air contaminated with acetone was periodically supplied. Again, after three days the charge’s samples were taken for microbiological tests. Afterward, the experiment was repeated by maintaining the temperatures of 30 and 35 °C in the filter.

Upon completion of experiments with acetone, the same tests were performed with other contaminants, i.e. butanol and toluene.

With the aim to determine the dependence of charge treatment efficiency on the amount of microorganisms, contaminant concentrations were recorded prior to and after charging. To determine the concentrations of contaminants, air samples before and after each cassette were taken in special sampling places and each measurement was repeated 3 times.

Air samples from the air duct are sucked via a stainless steel tube (d = 5 mm, l = 30 cm) into a clean gas pipette of 0.25 l at the velocity of 0.25 l/min. The sucking takes place for 5 minutes. Upon sucking completion, the pipette’s ends are via silicone hoses tightly stopped with glass plugs and the hoses are additionally tightened with Mohr’s pinchcocks. The samples are analysed on the same day.

The concentration of contaminants was determined with the gas chromatographer SRI 8610 No. 942. Upon turning the chromatographer on, the following parameters of the analysis process were determined: nitrogenous gas velocity – 30 ml/min, hydrogen gas velocity – 30 ml/min, air velocity – 200 ml/min, column thermostat temperature – 100 ± 2 °C, vaporizer’s temperature – 200 ± 5 °C, detector’s temperature – 200 ± 5 °C. Afterward, a zero line was tested. When the chromatographer is prepared for work, the gas pipette containing a sample is slightly heated up to 45 ± 5 °C and kept for 1 hour. 1 cm² of the analysed sample is sucked from it via a silicone hose into a clean medical syringe and passed though the vaporizer of the gas chromatographer. At least three chromatograms are recorded. The concentration of acetone is calculated according to them.

Microbiological tests of the charge’s samples are started with weighing 1 g of zeolite and 1 g of foam samples. Zeolite is crushed with a pestle in a sterile mortar. Foam is cut into small pieces. The samples of zeolite and foam prepared in the mentioned way are placed into a flask containing 100 ml of sterile distilled water and shaken for 10 min. The obtained suspension is diluted up to the concentration suitable for sowing. The first dilution corresponds to 1:100, the suspension is diluted by transferring 1 ml of
suspension to test-tubes, containing 9 ml of sterile water. Several dilutions –1:1000, 1:10000, 1:1000000 – are prepared. Prior to sowing, the test-tube is shaken. 1 ml from the selected diluted suspension is poured into a Petri dish and a diluted cooled agarized medium is poured over it. To obtain micromycetes, a mash medium with laevomycetini (100 mg/l), and to obtain bacteria – a nutrient agar were used.

![Fig 3. Bacteria (a) and micromycetes (b) obtained from zeolite samples when air contaminated with butanol was passed through the charge](image)

The composition of the nutrient agar: distilled water – 1000 g, agar – 15.0 g, peptone – 5.0 g, NaCl – 5.0 g, yeast extract – 5.0 g, meat extract – 1 g.

Sowing is repeated 3 times. Until the medium stiffens the dish is rocked to make the medium mix with suspension and uniformly spread over the dish bottom. Later Petri dishes with microorganisms are incubated in the thermostat. The raised cultures of microorganisms are given in Figure 3.

Petri dishes with bacteria were incubated in the thermostat for 2-3 days, those with micromycetes – 5-7 days at the temperature of 26 – 28 °C. The colonies of micromycetes and bacteria are calculated and the number of their springs per 1 g of the substance in question is determined. The number of live cells is calculated by multiplying the mean of the number of colonies in the dish by the dilution coefficient.

### 3. Investigation results

The charts present the dependences of the number of microorganisms on the air temperature maintained in the filter and the contaminant being used.

Data given in Figure 4 show that when air contaminated with acetone was passed through the mixture of zeolite and wood chips, the highest amount of microorganisms was obtained at 30 °C. At the temperature of 30 °C in the biomedium, the amount of bacteria in zeolite was 7.1 x 10⁴ cfu/g., that of micromycetes – 1.6 x 10⁵ cfu/g.

![Fig 4. The dependence of the amount of microorganisms found in zeolite on temperature when applying acetone](image)

The findings of the experiment show that when air is decontaminated from acetone using the charge composed of 10-12 mm zeolite granules, it was dominated by bacteria that perform the main role in decomposing organic compounds. The biggest amount of bacteria was obtained at the biomedium temperature of 30 °C. Therefore, this the temperature at the presence of which zeolite works best. A large amount of bacteria was discovered in form – 1300x10³ cfu/g. A bigger amount of bacteria in foam was predetermined by a lower density and a bigger area of sorption surface of this charge.

![Fig 5. The dependence of the amount of microorganisms found in foam on temperature when applying acetone](image)

As the data given in Fig 5 show, microorganisms adapt best in the charge composed of foam. However, it is a common knowledge that zeolite is distinguished by better properties of durability. Consequently, in order to achieve high efficiency of decontamination these two charges can be mixed up. In the meantime mycetes better develop at the temperature of 25 °C when the amount of micromycetes reached 10.6 x 10⁵ cfu/g. The smallest amount of microorganisms was recorded at the temperature of 20 °C. When temperature is rising the metabolism of microorganisms becomes more active. When reproducing, growing and breathing, organisms use nutrients and energy. Apart from that, when temperature is rising the velocity of substrate
fermentation reaction also increases. At the optimum temperature, very intensive metabolism takes place in the cells of microorganisms. Under favourable conditions one cell consumes 30-40 times more food-substrate than it weighs. Upon increasing the ambient temperature to 35 °C the number of microorganisms decomposing acetone starts decreasing. In zeolite as well as in foam the biggest amount of bacteria was discovered at the ambient (biomedium) temperature of 30 °C because psychrophilic and mesophilic microorganisms, that most often participate in the decomposition of organic compounds, are not capable of adapting at the presence of higher temperature.

![Fig 6](image1.png)

**Fig 6.** The dependence of the amount of microorganisms found in zeolite on temperature when using butanol

At 35 °C temperature, the amount of bacteria in zeolite fell and was $4.6 \times 10^4$ cfu/g, and that in foam – $9.5 \times 10^5$ cfu/g. When temperature rose, the amount of micromycetes also decreased.

When decontaminating air from butanol, bacteria played a more important role but at the temperature of 35 °C the charge composed of zeolite granules was dominated by fungi (micromycetes). Zeolite dominated by bacteria was distinguished by better treatment properties. At the temperature of 30 °C the amount of bacteria in zeolite reached $17.7 \times 10^3$ cfu/g. A bigger amount of microorganisms in foam is predetermined by the charge’s better properties to sorb water saturated with salts and biogenic elements. Bacteria were predominant in both charges at the temperature of 30 °C. A smaller amount of bacteria was discovered at the temperatures of 20 and 35 °C.

When decontaminating air from butanol a bigger amount of bacteria was discovered in foam (Fig 7). At the temperature of 30 °C $1300 \times 10^3$ cfu/g of bacteria were discovered. The amount of bacteria in zeolite reached $177 \times 10^3$ cfu/g. The amount of fungi was also predominant in foam when decontaminating air from butanol. But upon raising the biomedium temperature up to 35 °C, the amount of mycetes in both foam and zeolite becomes equal and reaches nearly $260 \times 10^3$ cfu/g.

![Fig 7](image2.png)

**Fig 7.** The dependence of the number of microorganisms found in foam on temperature when using butanol

Even though butanol is distinguished by a good coefficient of sorption, a big surface area and porosity, due to its composition it has smaller resistance to the effect of microorganisms. Fungi (micromycetes) have the major influence on the colonisation and destruction of foam containing polymeric substances. At the beginning micromycetes feed on nutrients present on the charge’s surface. The bigger is the amount of nutrients on such surface the micromycetes have to assimilate, the better growth and development effect is achieved. The surface of contact of micromycete’s mycelium with the charge increases and nutrition intensifies. A fungus forms mycothalli. A new generation emerges. The substance on which a fungus settles starts decaying, losing weight, its appearance and operational properties change. When foam decays new metabolites emerge and they combine with metabolites that evolve from fungi.

Decontaminating air from toluene the biggest amount of bacteria was achieved when the temperature of biomedium reached 35 °C. Consequently, zeolite becomes dominated by bacteria that are better adapted to thermophilic conditions (Fig 8.).

![Fig 8](image3.png)

**Fig 8.** The dependence of the amount of microorganisms found in zeolite on temperature when applying toluene
In the meantime foam was distinguished by good decontamination properties when the biomedium temperature was 25 °C. The physical properties of foam are more favourable for the bacteria that are better adapted to mesophilic conditions.

When decontaminating air from toluene, charges composed of zeolite and foam are dominated by bacteria. The comparison of charges shows that the foam charges were distinguished by a bigger growth of bacteria than the zeolite ones. The biggest difference was recorded at the temperature of 25 °C and reached 1500 x 10³ cfu/g. The amount of fungi playing a smaller role in air treatment was significantly lower than that of bacteria.

The capacity of microorganisms to oxidise the selected contaminants also depends on the degree of biological activation of the filter’s charge.

As the data given in Figure 10 show, the number of microorganisms after activation significantly increased. Prior to activation the number of bacteria in the control sample of zeolite hardly reached 1.4 x 10³ cfu/g.

Microorganisms can develop well in a charge of inorganic origin, i.e. zeolite. The biggest amount of microorganisms was achieved when decontaminating air from butanol at the temperature of 30 °C. The amount of bacteria in the sample of zeolite reached 17.7 x 10⁴ cfu/g. When decontaminating air from acetone and toluene the biggest amount of bacteria was obtained at the temperature of 30 °C.

Bacteria can reproduce in a charge composed of foam. The experimental findings show that a big amount of microorganisms is discovered when decomposing such organic compounds as acetone, butanol or toluene. When decontaminating air from acetone or butanol one gram of foam contained 1300 x 10³ cfu/g of bacteria at the supplied airflow temperature of 30 °C. When decontaminating air from toluene, the biggest amount of bacteria in foam was recorded at the temperature of 25 °C and amounted to 1530 x 10³ cfu/g.

A bigger amount of micromycetes was recorded in foam. When decontaminating air from acetone, the amount of fungi is 1060 x 10³ cfu/g at the temperature of 25 °C. When decontaminating air from butanol, the growth of fungi was recorded with an increase in temperature of a supplied airflow. At the temperature of 35 °C the amount of fungi in foam reaches 260 x 10³ cfu/g. But when decontaminating air from toluene a bigger amount of fungi was discovered at a lower temperature. At the temperature of 20 °C the amount of fungi (micromycetes) reaches 263 x 10³ cfu/g.

The activity of microorganisms increased upon mixing zeolite and foam with wood chips. This can be proved by a small amount of microorganisms found in control samples of zeolite and foam taken before activation.

During experimental tests the concentrations of contaminants and their dependences on the air temperature in the biofilter were determined. According to the experimental findings, the treatment...
efficiency of the charge was for the most part predetermined by bacteria. Even though the amount of microorganisms is much smaller in a charge composed of natural zeolite than in a foam charge but the treatment efficiency compared to a charge composed of foam and wood chips remained similar. The highest treatment efficiency was achieved when decontaminating air from butanol. An activated charge of 150 mm layer composed of zeolite and wood chips sorped and decomposed around 112 mg/m³ of butanol.

The highest efficiency of air decontamination from acetone, butanol and toluene was achieved at the temperature of 30 °C. Toluene was worst sorped by zeolite. Toluene is a less soluble compound compared to acetone or butanol and, therefore it is worse soluble in water. Rather low coefficients of approximation show that the treatment efficiency of a charge depends not only on the amount of microorganisms contained therein but zeolite retains part of contaminants amount in its pores.

According to literature, the best decomposition of contaminants is achieved at the temperature of 20 – 40 °C [18]. During the experiment the biggest amount of bacteria was achieved at the temperature of 30 °C when decomposing acetone and butanol. Toluene was decomposed best at the temperature of 25 °C. Consequently, when using activated charge composed of foam and wood chips toluene is better decomposed by the microorganisms of psychrophilic origin to which belong many cultures of the Pseudomonas genus. High coefficients of approximation show a high dependence of decontamination efficiency on temperature, i.e. on the amount of microorganisms in a charge composed of foam and wood chips.

4. Conclusions

1. The process of organic compounds destruction was dominated by bacteria. Bigger amounts of bacteria were also discovered in the control samples of zeolite and foam. Zeolite contained 1.4 x 10³ cfu/g of bacteria and only 200 cfu/g of micromycetes.

2. A significantly bigger amount of microorganisms was discovered in foam and reached 7.3 x 10⁴ cfu/g, and zeolite contained only 1.6 x 10³ cfu/g of microorganisms. A bigger amount of microorganisms in foam was predetermined by a larger surface area and porosity of foam as well as lower density of the charge.

3. The best activity of bacteria was achieved when decomposing organic compounds at the temperature of 30 °C. Psychrophilic and mesophilic microorganisms, such as Pseudomonas, which synthesise contaminants well, become more active at such temperature.

4. The decomposition of butanol was the most efficient. When passing it through a 150 mm layer of zeolite and wood chips at the charge’s temperature of 30 °C the concentration of butanol fell to 84 mg/m³. Such change in concentration was also predetermined by the biggest established amount of microorganisms – 17.7 x 10⁴ cfu/g.

5. The decomposition of contaminants depends not only on temperature but also on the type of a charge. Higher decomposition efficiency of acetone and toluene in zeolite was achieved at the temperature of 30 °C, in the meantime the
concentration of toluene in foam decreased the most at the temperature of 25 °C. Upon increasing temperature to 35 °C the destruction of contaminants becomes slower and the treatment efficiency of charges starts decreasing.

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