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Thermophilic Biofiltration: Start-up phase optimization for VOCs and odorous compounds treatment utilizing modified plastic carrier material

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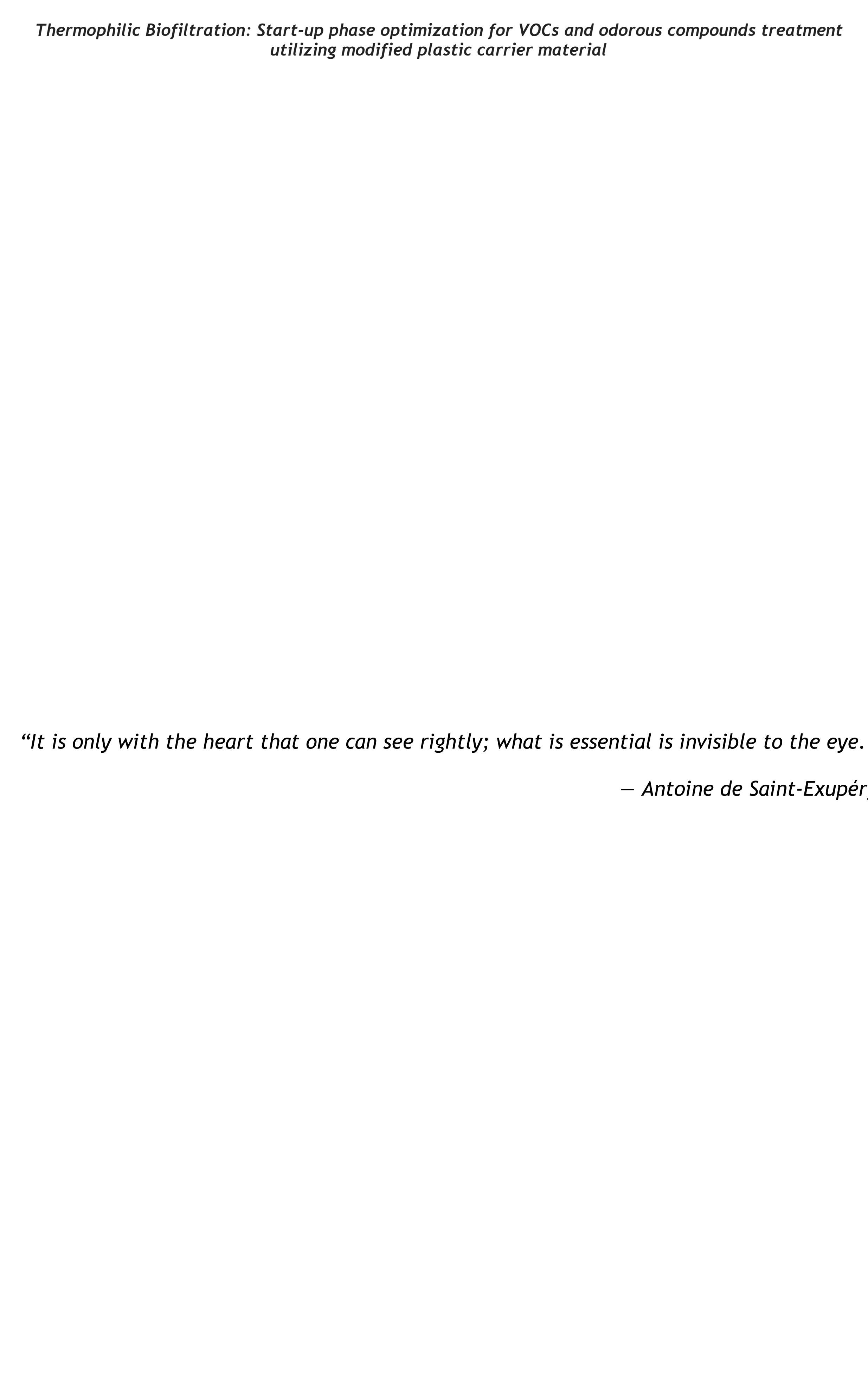

"It is only with the heart that one can see rightly; what is essential is invisible to the eye."

— Antoine de Saint-Exupéry

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Abstract

Biofiltration has been proved to be a low cost and environmentally friendly alternative to several traditional methods for odorous and volatile organic compounds treatment from waste air stream. The general mechanism of action includes the pollutants' transference from the air stream into a porous carrier material, where immobilized microorganisms will degrade them into non-harmful compounds. Its application, however, has been limited to the treatments occurring in mesophilic operational conditions. Biofilters working under high temperatures represent a convenient solution for waste air treatment from industrial sources where gaseous streams are released in high temperatures, such as pulp and paper, tobacco and other industries.

The scope of this study was to investigate the possibility of removal of volatile organic compounds and odorous compounds from waste air under thermophilic conditions, utilizing a biodegradable foam as the carrier material. A laboratory scale biotrickling filter was set up at a temperature of 55ºC to remove a range of selected pollutants. The pollutants propionic acid and ethyl acetate proved to be successfully degraded, with EC of 27.01 $g.m^{-3}.h^{-1}$ and 28.31 $g.m^{-3}.h^{-1}$, respectively, with a removal efficiency around 90%, for inlet concentration equal to 0.66 $g.m^{-3}$. Higher elimination capacity of 47.73 $g.m^{-3}.h^{-1}$ and 49.02 $g.m^{-3}.h^{-1}$ was achieved for these compounds, when inlet concentration raised to 1.10 $g.m^{-3}$. At this condition, their removal efficiency was around 95%.

Key Words: Thermophilic Biofiltration, Volatile Organic Compounds, Odorous Compounds, Biodegradable Carrier Material.

Resumo

Biofiltração é uma alternativa ecológica e de baixo custo ao vários métodos tradicionais de tratamento de ar contamidado por compostos orgânicos voláteis e odoríferos. O mecanismo geral de acção inclui a transferência de polutantes da corrente de ar para um material de suporte poroso, onde microrganismos imobilizados irão degradá-los em compostos não nocivos. A sua aplicação, no entanto, tem sido limitada aos tratamentos em condições operacionais mesofílicas. Biofiltros que operam sob temperaturas elevadas representam uma solução conveniente para o tratamento do ar residual de origem industrial, onde correntes gasosas são liberadas em altas temperaturas, tais como em industrias de celulose e papel e de tabaco.

O objectivo deste estudo foi investigar a possibilidade de remoção de compostos orgânicos voláteis e odoríferos do ar residual em condições termófilas, utilizando uma espuma biodegradável como material de enchimento. Um biofiltro de escala laboratorial foi construído a uma temperatura de 55ºC, para a remoção de uma variedade de poluentes seleccionados. Os poluentes ácido propiónico e acetato de etilo provaram ser degradados com sucesso, com capacidade de eliminação de 27.01 $g.m^{-3}.h^{-1}$ e 28,31 $g.m^{-3}.h^{-1}$, respetivamente, e com uma eficiência de remoção de cerca de 90%, para a concentração de entrada igual a 0,66 $g.m^{-3}$. Uma maior capacidade de eliminação de 47.73 $g.m^{-3}.h^{-1}$ e 49.02 $g.m^{-3}.h^{-1}$ foi obtida, respectivamente, quando a concetração de entrada aumentou para 1.10 $g.m^{-3}$. Nestas condições, a eficiência de remoção de ambos foi a volta dos 95%.

Palavras Chave: Biofiltração termófila, Compostos Organicos Voláteis, Compostos Odoríferos, Material de Enchimento Biodegradável

Declaration

Declare, under oath, that this work is original and that all non-original contributions were properly identified by the source.

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LIST OF SYMBOLS

EBRT – Empty Bed Residence Time

EC – Elimination Capacity

EPS - Extracellular Polymeric Substance

OD – Optical Density

RE – Removal Efficiency

VOCs – Volatile Organic Compounds

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1 Introduction

Over the past decades, large quantities of industrial pollutants, including volatile organic compounds (VOCs) and odorous compounds, have been released into the environment. These compounds are not only harmful to human health, but also hazardous to the environment, contributing to photochemical smog and secondary particle formation.

As a result, there is an increasing demand for air pollution control, through stringent environmental regulation, leading the polluting industries to adopt effective treatment for air pollution control.

Several technologies have been developed for treatment of air pollution, including physical-chemical treatments (adsorption, incineration and scrubbing) and biological technologies (Biofilters, Biotrickling filters). Biofiltration is a cost-effective and environmentally friendly technique that has been successfully used for the treatment of waste gases containing odorous and volatile organic compounds. It utilizes microorganisms, attached to a porous support media, where the pollutants are transferred to and degraded into non harmful compounds, such as carbon dioxide and water.

Despites its advantages, biofiltration treatment has been limited to operations at mesophilic temperatures (20ºC to 35ºC). Nonetheless, effluents originated from industrial processes can be released in high temperatures (40ºC to 70ºC), such as pulp and paper manufacturing, tobacco industry and coating processes, leading to the necessity, prior to the biological treatment, to cool down the streams, which is a highly costly procedure. An alternative is the use of thermophilic biofilters, which will not only be more economical but will also extend the applicability of biofilters.

The scope of this project is to evaluate the treatment of air pollutants by thermophilic biofiltration utilizing a biodegradable packing material.

1.1 Company Presentation

DEKONTA is one of the leading Czech waste management, consulting and engineering companies offering a comprehensive package of environmental services: hazardous waste treatment and disposal; remediation of contaminated sites; nation-wide 24-hour environmental emergency response service; environmental consulting and laboratory service.

The portfolio of the company includes biofiltration and other types of catalyst waste air treatment equipment. Currently, the company offers three types of biofilters: 1) bed biofilters, 2) biofilters with air scrubbers and 3) biotrickling filters with moving bed.

DEKONTA has a professional research unit developing innovation technologies for the company, also in the field of bioremediation of polluted sites, water and air. The company dispose of biotechnological laboratory, where new types of biopreparates are developed.

1.2 Work Contribution

There is a high demand for the biological treatment for hot waste air on the European market. The most used technologies nowadays need cooling prior to treatment, which causes higher energy consumption.

Business opportunity is presented by eliminating needs of cooling devices and decreasing procedure costs, without decreasing process efficiency. The special innovation is presented in the new type of biodegradable carrier material, which has the ability to adsorb other contaminants (e.g. nitrogen, phosphorus), is biodegradable and compostable.

1.3 Thesis Organization

This work consists of the following chapters:

- State of the Art;
- Material and Methods
- Discussion of Results;
- Conclusions

The section of State of Arts it describes different concepts necessary for the comprehension of this work. It starts with an introduction to volatile organic compounds and odorous compounds, their sources, and their implacability in human's health as well as to the environment. Followed by the description of air pollution control technologies type, highlighting biofilters.

Posterior, it introduces biofilm, the key element of the pollutant's biodegradation in biofilters. It is explained what is a biofilm, its structure, how it is formed and a briefly introduction to cell-to-cell communication within the biofilm.

Next, it introduces biofiltration and its advantages and the two main types of bioreactors for the treatment: Biofilter and Biotrickling filter, as well as their mechanism of operation and the comparison between them. It is then introduced the thermophilic biofiltration, showing its

importance, and description of studies where pollutants were treated under thermophilic conditions, attesting its advantages in comparison to mesophilic treatment, but also explaining its limitation. It is then mentioned the types of carrier material, where the microorganisms creates the biofilm.

In the chapter of the material and methods it is described all laboratory procedures performed, including, the tests to evaluate the growth curves of the strains chosen for this project, as well as their capability to create a biofilm on the selected carrier material, and the initial tests in the biofilter.

The chapter of Discussion of Results, all the results of each test carried out during the work are presented and discussed.

Finally, in the chapter of Conclusions, it is presented the main conclusions of this work.

1.4 Main tasks

The main tasks of this project are listed below.

- Evaluate the best range of pollutants concentration for biodegradation by the strains BIOF and HR15;
- Evaluate the possibility of using the foams as inoculating material;
- Evaluate the best packing material between three foams tested;
- Efficiency evaluation of the pollutants' biodegradation in a thermophilic biotrickling filter utilizing the biodegradable carrier material.

2 State of Art

2.1 VOCs and Odorous compounds

Volatile organic compounds are a large group of odor compounds with different physical-chemical properties, but show similar behavior in the atmosphere and are responsible for the production of pollutants known as photochemical oxidants, principally ozone (Santos, 2010). It is important to notice that not all odor compounds are VOCs, but odours are often indicative of volatile organic compounds' presence. They are caused by mixtures of highly volatile compounds with very low threshold detection limit that are generally in small concentrations (Koppmann, 2007; Vankeerbergen, 1994).

Examples of odorous compounds associated with industrial, municipal and agricultural sources are presented below.

Table 1: Example of odorous compounds associated with several sources (based on Australian Laboratory Services – ALS Global)

Souce	Odorous Compounds
Wastewater treatment	Acetaldehyde, ammonia, butylamine, ethylamne, methylamine and trimethylamine
Biosolids and composting	Reduced sulfur compounds, ammonia, dimethylamine, trimethylamine, organic acids (acetic, butyric, valeric), 2-pentanone, terpenes
Livestock opeations	Hydrogen sulfide, ammonia, dimethyl sulfide, dimethyl trisulfide, carboxylic acids, phenols
Petroleum refining	Organic sulfides, aldehydes, phenolic compounds
Pulp and Paper	Dimethyl sulfide, dimethyl disulfide, hydrogen sulfide, terpenes

According to the European Environment Agency, volatile organic compound is any organic compound having at 293.15 K a vapour pressure of 0.01 kPa or more, or having a corresponding volatility under the particular conditions of use and includes chemical compounds such as methane, benzene, xylene, propane and butane. The United States Environmental Protection Agency defines these compounds as any compound of carbon, excluding carbon monoxide,

carbon dioxide, carbonic acid, metallic carbides or carbonates, and ammonium carbonate, which participates in atmospheric photochemical reactions and has vapour pressure exceeding 0.1 mL of mercury (mm Hg) at standard conditions (20ºC and 760 mm Hg).

The source of VOCs emissions can be divided into Anthropogenic (related to human activity) and Biogenic (from natural source). The main anthropogenic sources are combustion, such as fossil fuel and biomass burning (human origin), the usage of solvent and emissions from industrial processes. The biogenic sources include biomass burning (from natural forest fire), soil, trees, volcano activities and ocean (Koppman, 2007).

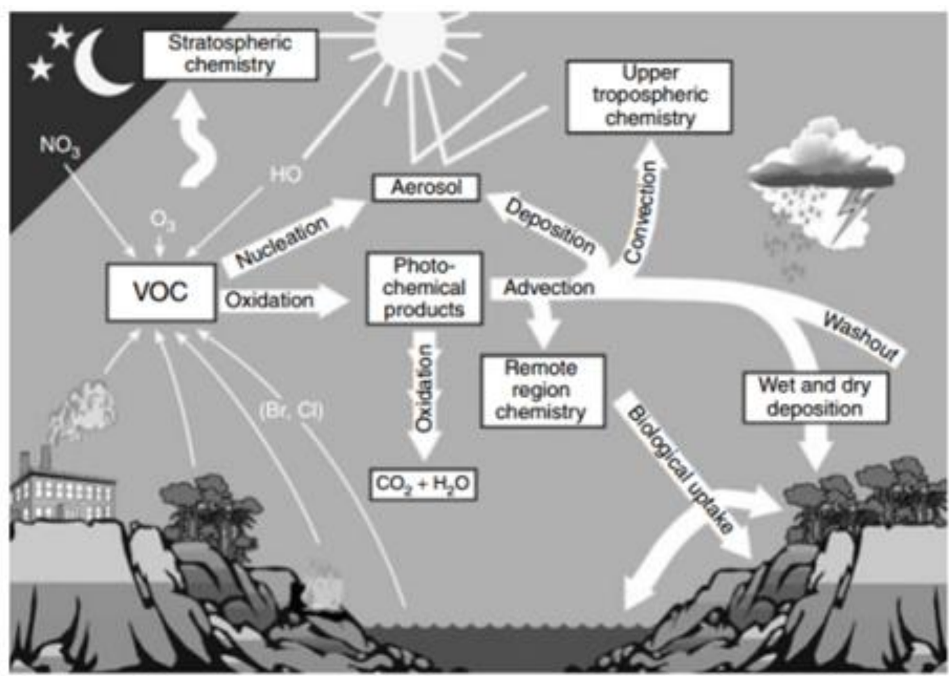

Figure 1: Sketch of the various processes which determine the fate of VOC in the atmosphere (koppman, 2007)

According to Koppman, the estimated the global natural VOC emissions is around 1100 TgC/year, whereas the VOC emission from anthropogenic source is around 186 TgC/year.

Volatile organic compounds (VOCs) and odorous compounds present problem to human and environmental health. When release to the atmosphere become potential air pollutants, being able to affect the nervous and breathing systems, and also lead to cancer. In addition, VOCs contribute to global warming, stratospheric ozone depletion and tropospheric ozone formation, which causes the urban smog (Margesin and Schinner, 2001), as described in the equation bellow (Delhomenie and Heitz, 2005).

$$\text{Sunlight} + \text{VOC} + \text{NOX} + \text{air} \rightarrow \text{photosmog}$$

Several air pollution control technologies (APCT) were developed for the removal of odorous compounds and VOCs from gaseous emissions. They include adsorption, absorption, membrane process, catalytic oxidation, incineration, electronic coagulation and others. These mechanisms are very effective at reducing the concentration of these compounds in air, but, besides being costly, they can generate secondary waste streams (Bajpai, 2014).

A recent and popular alternative for high volume of polluted air with low concentration of pollutants is the biological treatment, which uses microorganism to biodegrade contaminants and will transform them into less harmful, non-hazardous substances (Margesin and Schinner, 2001). This process, known as biofiltration, is done in bioreactors such as biofilters and biotrickling filters.

They have the advantage of being cost effective, environmentally friendly, do not generate NO_x, SO_x, or secondary pollutants, and are highly effective, being able to remove odour from polluted air up to 99% and are reliable (Fulazzaky et al., 2014).

Comparison of different methods of air pollution treatment is showed in the figure 2.

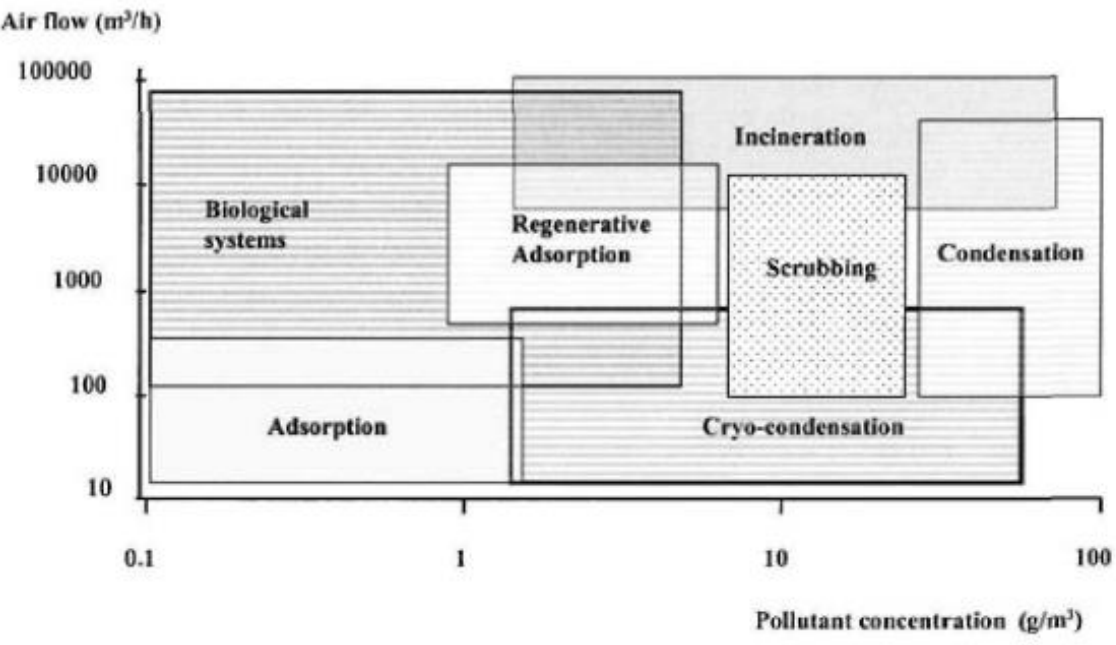

Figure 2: Comparison of methods for decontamination of air-polluted emissions. (Revah and Auria, 2004)

2.2 Biofilm

The key element of the pollutant's biodegradation is the biofilm. It can be described as a aggregates of cells attached to a surface surrounded by a matrix of extracellular polymeric substance (EPS), which they produce, and foreign substances, such as adsorbed molecules and

small abiotic particles (Melo, 2003). It can be formed by a single bacterial species, but in nature, biofilms is almost always formed by a rich mixture of many bacterial species as well as other microorganism's species (Cunningham et al., 2008).

It is acknowledge that cells in biofilms, also known as sessile cells, have lower microbial growth rate than planktonic cells, which are cells in suspension (Donlan, 2002). Despite of it, the biofilm formation is very important for the microorganisms since the proximity it provides leads to the creation of protective network from environmental aggression, such microbial agents (Donlan and Costerton, 2002).

The microenvironment created will affect not only the microorganisms' metabolism but also their genome, since horizontal gene transfer is facilitated by the proximity, leading sessile cells to transcribe genes that planktonic of the same species does not (Donlan and Costerton, 2002; Melo, 2003 and Jabra-Rizk et al., 2006 Flemming et al., 2007).

Biofilm has a very heterogeneous structure and its architecture not only differs from cell community but also from time. In older biofilms, for instance, the biopolymers build bridges between the microcolonies and enhance the fixed biomass density. In general, the structure of biofilms includes cell clusters, channels, void areas and streamers (Melo, 2003).

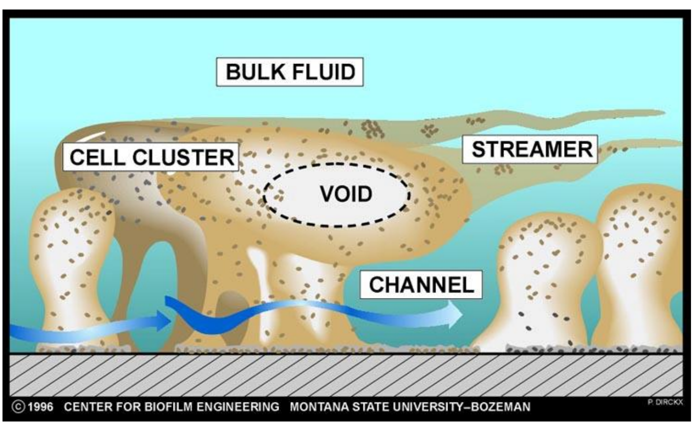

Figure 3: Biofilm Structure (Based on Center for Biofilm Engineering – Montana State University)

- *The Cell clusters*: Are the microcolonies and contain the polymeric matrix. It can be consisted of different layes containing different microbial species, composition of the matrix and densities of active microorganisms.
- *Channels:* Through which the medium liquid carring the nutrients flows,
- *Void areas*: Areas that are not or no longer colonized with cells
- *Streamers*: Are created by the bulk fluid flow (Cunningham et al., 2008; Melo, 2003).

The cells in biofilm, as it was already mentioned, are surrounded by extracellular polymeric substances excreted by them, and are important keys for the biofilm start-up. Its structure is made of 1 to 2% of proteins (including enzymes), less than 1% of DNA and RNA, 1 to 2% of polysaccharides and up to 97% of water content. The amount of each component depends on the type of microorganism and it can be enzymatically modified in response to changes in the environmental conditions, such as nutrient availabily, pH, temperature, etc (Jamal et al., 2015).

These substances have several functions in the microbial biofilm. They provide the initial adhesion of the planktonic cells, contribute to the attachment of the biofilm to the surface and keep the microorganisms attached to each other allowing the cell-cell communication. Besides, they maintain the microenvironment hydrated and create a protective barrier that will confer resistance during infection and tolerance to various antimicrobial agents, such as biocides and antibiotics (Muhsin et al., 2015; Flemming et al., 2007; Manuel, 2007).

The formation of Biofilm is a process where the cell switch from a planctonic to a sessile life resulting in a structured community. It involves several steps, which are decribed bellow.

- **Formation of Conditioning Film**

Conditioning film is a very thin layer that consists of proteins and polysaccharide molecules that will be the place of the first attachment of cells. It is formed by adsorption of the organic substances on a substrate at the moment it contacts the liquid phase (Hohmann et al., 2015). The nature of the conditioning films may be quite different depending on several conditions, such as, the kind of environment, the surface in which it is exposed to and the rate at which it is formed (Brenner-Weiß et al.,2015; Chamberlain, 1992).

- **Initial Adhesion**

After the initial conditioning film is stablished, the microbial cells are transported from the liquid medium to the solid surface. Initially the adhesion of the bacteria is reversible and it occurs as a result of the van der Walls attraction forces, electrostatic forces and hydrophobic interactions. This is a weak attachment and the cells still exhibit Brownian motion (Characklis et al., 1993).

The contact with the surface the molecules from the conditioning film forms polymeric chains, which will interact with the EPS matrix on the surface of microorganisms. It will stabilize the cell adhesion by forming strong bridges, and subsequently the irreversible adhesion. This adhesion is characterised by dipole-dipole interactions, hydrogen, ionic and covalent bonding and hydrophobic interactions. The transition time between from the reversible to the irreversible attachment depends on the organism and the physical conditions presented (Melo, 2003; Characklis et al., 1993; Forsythe, 2010).

- **Microbial growth**

The cells, that are adsorbed on the conditioning film, replicate, grow and produce EPS, leading to the built of single species microcolonies followed by the adhesion of other cells and abiotic material (Melo, 2003; Donlan and Costerton, 2002).

During this phase, detachment of the microorganisms occurs, as a parallel process, however, it happens at a lower degree in comparison to their growth. Consequently, the rate of biofilm formation results from the competition between the rate of microbial growth and the detachment process (Melo, 2003).

- **Biofim maturation**

The continuous attachment and growth of bacterial cells along with the EPS formation leads to the three-dimensional growth of the biofilm. Different factors will determine the thickness and architecture of the mature biofilm among which: species composition of the community, the nutrient conditions and the rate of flow of the liquid passing through the biofilm (Beloin et al., 2008; Forsythe, 2010). According to Cunningham et al.(2008) low nutrient conditions in the medium leads to the creation of a robust biofilm with complex architecture, while in reach medium, low growing biofilms and not as well attached to the surface are observed.

- **Detachment**

Detachment is any mechanism that leads to the separation of individual cells or cell masses from the substratum at which the biofilm is attached. This phenomenon can be the result of shearing forces or sloughing (Cunningham et al., 2008).

Shearing forces refers to the continue removal of small portions either by abrasion which is due to repeated collisions between substratum particles, or erosion, which is the continuous loss of biomass or cell prodigy from the upper layers of a biofilm (Lappin-Scott and Costerton, 2003).

Sloughing is intermittent detachment of large pieces of biofilm resulted from the conditions within the biofilm such as nutrient or oxygen depletion or to some dramatic change in the immediate environment of the biofilm (Bakke, 1986; Howell and Atickson, 1976). This phenomenon usually occurs with thicker biofilms developed in nutrient-rich environments (Characklis et al., 1993).

According to Cunningham et al. (2008) there is increasing evidence that biofilms initiate detachment on their own. Some enzymes, for instance, alginate lyase, hydrolyses the matrix, which the biofilm is encased leading to detachment of substantial portion of it (Kaplan, 2010). Other approaches are also used to accomplish biofilm dispersion, such as, ending the synthesis

of the biofilm EPS compounds and disrupting noncovalent interactions between matrix components. Solano et al. shows that recent evidences indicates that many bacteria species use the Quorum Sensing system to perform it.

Quorum sensing is a cell to cell communication mechanism used by the bacteria to monitor their population density by measuring the accumulation of signalling molecules, known as the autoinducers, also secreted by them (Solano et al., 2014 ; De Kievit and Iglewski, 1999). When the cell density increases, the concentration of these molecules also increases and its accumulation stimulates a response, through the induction or repression of target genes (Rabin et al., 2015).

2.3 Biofiltration

Biofiltration, as mentioned before, is a cost effective, environmentally friendly, highly effective and reliable alternative to biological treatment of waste gas and control of volatile organic and inorganic compounds as well as other toxic and odorous compounds (Soccol et al., 2003).

The mechanism of treatment involves the polluted air to flow through the porous packed bed of the biofilter on which a mixed culture of microorganisms is immobilized. The pollutants are transferred, along with oxygen, from the gaseous phase to the biofilm, in which are subsequently biodegraded into non harmful compounds, such as carbon dioxide and water, or are incorporated into biomass (Jeong et al., 2006). The figure 4 shows the mechanism of biodegradation that occur in biofilters.

In the treatment of waste air, a most effective filter to be utilized is the Biotrickling filter. It is a newer technique, in comparison to biofilters, which allows higher elimination capacities for a larger range of pollutants. Because it is more costly method than the conventional bioreactor, it is usually applicable to the purification of waste air streams that are more difficult to treat, for instance, halogenated organic compounds (Cox and Deshusses, 1998 ; Plaggemeier and Lämmerzahl, 2000).

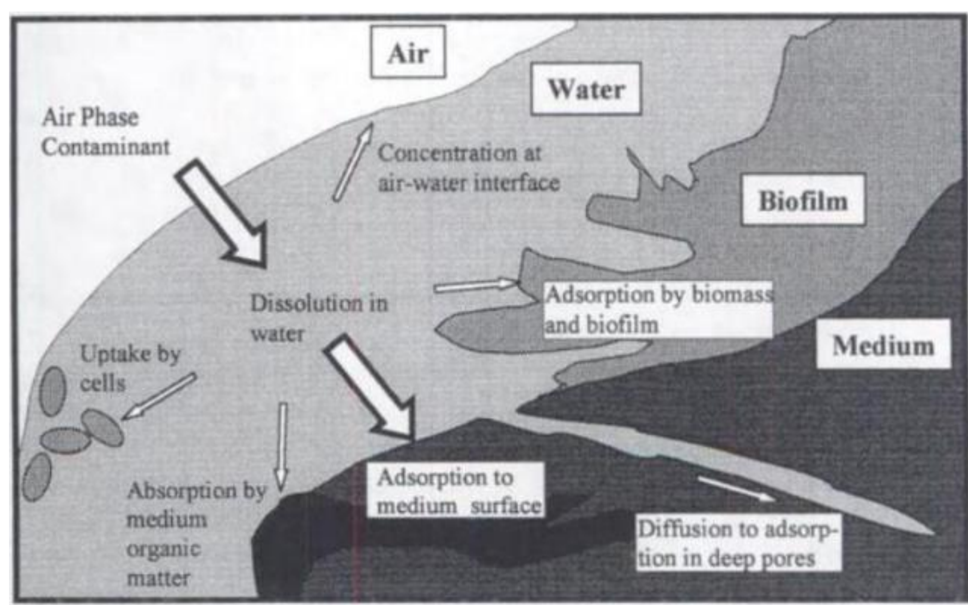

Figure 4: Mechanism of biodegradation in Biofilms

The differences between these two bioreactors are the type of packing material and consequently the presence of liquid phase in the biotrickling filters.

The filling of the biofilter is a bioactive media that provides nutrients (nitrogen and phosphorus trace minerals) for biodegradation. This packing material has also sufficient water retention capacity to maintain its bioactivity by using humidified air, and so the introduction of continuous fluid flow is not necessary (Govind and Narayan, 2005).

Biotrickling filters, moreover, are mostly packed with inert material, such as plastic packing or synthetic foam. This type of material do not contain the nutrients necessary to the biodegradation, and have no or low water retention capability, being necessary to provide continuous flow of liquid that will transport the nutrients needed, buffer any pH changes that may occur and allow biomass excess to be sloughed-off. Additionally, since the packing matter does not contain any microbial populations, inoculation in the beginning of the process is required (Govind and Narayan, 2005).

The mechanism of operation of the trickling filters is similar to biofilters, but instead of the gaseous phase be directly transferred to the biofilm, the air stream is firstly solubilized in the liquid phase. The fluid is continuously recirculated in co- or counter current flow through the packing and according to Cox et al. (2001) since the trickling liquid is recycled, co-current flow (air in a down flow motion) is preferred over counter-current flow. In the Counter-current system, the air can pick up pollutant from concentrated liquid just before leaving the bioreactor, reducing the efficiency of the treatment. However, no experiment have succeeded to show a significant difference between those two (Cox and Deshusses, 1998).

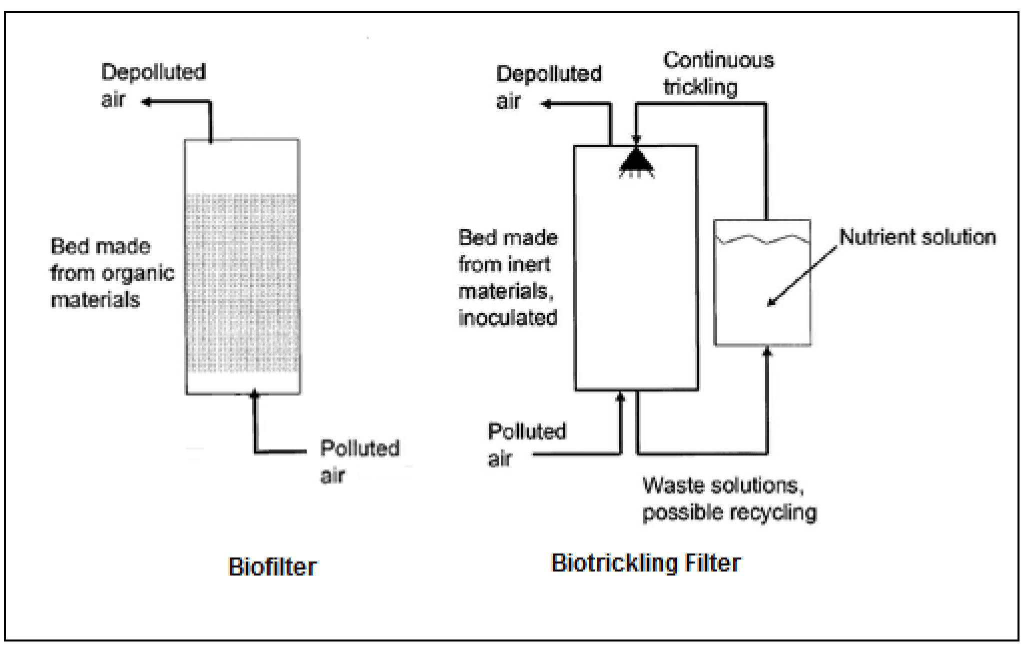

Figure 5: Generic schematics of Biofilter and Biotrickling filter (based on mudliar et al., 2010)

2.3.1 Thermophilic Biofiltration

The treatment in biofiltration for removal of odorous and volatile organic compounds from waste air has been often done in mesophilic temperature range. However, many waste streams from paper, tobacco and other industries are released in at significantly higher temperatures (40-75ºC), which leads to the necessity, prior to treatment, to cool down the waste gases, which is a costly procedure, especially if they are saturated with water (Luvsanjamba et al., 2008).

An alternative for this treatment is the use of thermophilic microorganism for degrading those pollutants, which will not only save resources but will also extend the applicability of the biofilters.

There have been only few researches on the thermophilic biodegradation of waste gas, but most of them were able to verify that VOCs can be treated in thermophilic biofilters, and some of them with higher removal rates in in comparison to the mesophilic ones. For instance, Matteau and Ramsay (1997) demonstrated that toluene biodegradation rates were around 110 $g_{toluene}.m^{-3}.h^{-1}$ at the temperature near 50ºC, whereas in mesophilic conditions, the maximum rate achieved was around 89 $g_{toluene}.m^{-3}.h^{-1}$. Dhamwichukorn et al. (2001) showed that both methanol and *α*-Pinene at a residence time of 18.24 min had removal rates over 95% in biofilter at 55ºC. Kong at al. found that methanol at removal rates over 100 g $m^{-3}.h^{-1}$ and α-Pinene at rates up to 60 g m^{-3} h^{-1}.could be degraded at temperatures up to 70ºC and 55ºC respectively. Luvsanjamba et al. (2007) showed that isobutyraldehyde and 2-pentanone had

higher elimination capacities when treated in thermophilic condition (52ºC) than in mesophilic conditions (25ºC) in biotrickling filters. Also observed that in higher temperatures foam formation, biomass accumulation and organic acid production did not occur or were less severe than in the reactor working in room temperature. Cho et al. (2007) studied that benzene and toluene gases can be degraded in thermophilic biofilter utilizing yeast extract having higher elimination capacity (1.650 $g \cdot m^{-3} \cdot h^{-1}$) in comparison to mesophilic biofilters.

As shown in these studies, bioreactors operating in thermophilic conditions have higher elimination capacities and lower biomass accumulation in the biofilter bed, which avoids clogging of the packing material. However, unlike mesophilic temperatures, some problems are often observed on thermophilic biofilters, such as the limitation in mass transfer. With the increment of temperature, Henrys coefficient will also increase resulting in a lower driving force for interphase mass transfer (Luvsanjamba et al., 2008).

Another occurring problem in thermophilic biofilters is the acceleration of the organic material degradation causing a higher-pressure drop and bed compaction (Cox et al., 2001). Additionally the increase of temperature leads to higher water evaporation rate, which will hamper the maintenance of proper and homogeneous bed moisture content (Kennes and Veiga, 2001). Therefore the use of biotrickling filters for thermophilic treatment is, more favourable, since liquid is recirculated during the whole process and the packing material has a different nature than the biofilters.

2.3.2 Carrier material

The carrier material, also called biofilter bed, packing material or also medium, is essential for the operation of bioreactors, since it gives support to the microbial biofilm and allows the polluted waste gas to interact with the degrading microorganisms, oxygen and water (Bajpai, 2014).

The selection of the carrier material is an important factor and it must have desirable properties that includes: high specific surface area, for the development of the biofilm as well as for the adsorption of contaminants , high porosity and proper pore size , that will facilitate homogeneous distribution of gases and will prevent head loss and clogging problems; good water retention as well as good moisture content (usually within 30%to 60% by weight), to avoid bed drying; presence and availability of essential nutrients as well as diversified microflora, in case of organic material. If the packing material is inert, it should be inoculated prior to treatment. The carrier material should also be physically stable and well-structured in order

to ensure that the medium does not compact or degrade during the process (Bajpai, 2014; Mudliar et al., 2010; Park, 2004; Kumar et al., 2011).

As mentioned before, the most used packing material in biofiltration are organic (peat, wood chips and compost) and inert (ceramic, activated carbon, foam plastic packing or synthetic foam). Moe et al. (2000) studied the possibility of utilizing polyurethane foam as a carrier material. The results showed that the foam has high porosity, suitable pore size, low density, low head loss, and ability to sorb water. Additionally, it can overcome problems such as clogging, and has favourable head loss characteristics compared to other packing material.

3 Material and Methods

3.1 Bacterial strains

Previous the beggining of this project, Dekonta initiated a research to isolate thermophilic bacterial strains to be used in this new work to be developed. They isolated thermophilic organisms from six locations in CZ: the waste air pipe outlet from animal feed factory (Kromeriz, CZ); the waste air pipe outlet from paper mill (Sumperk, CZ); waste air pipe outlet from plastic manufacture (Stribro, CZ), the burning slag heap Ema (Ostrava, CZ); the biofilter in black coil mine (Paskov, Ostrava, CZ).

After one month of growth in Erlenmeyer flasks with addition of VOCs as a source of cabon and yeast extract, pure cultures were isolated. Every strain was tested by Gram's method and then identified by 16S rRNA gene sequence analysis, by an external company. Based on bacterial growth tests the 2 most suitable strains were chosen for further testing for inoculation in Bioreactor: strains BIOF and HR15 They were then lyphilized in a microbiological lab before being sent to Dekonta.

Table 2 shows all the strains isolated from the six locations and the ones in bold were the strains selected to this study.

Table 2: Strains isolated by Dekonta

Strain designation	Identification
BIOF	***Geobacillus caldoxylosilyticus***
BIOF-2	*Aeribacillus pallidus*
BIOF-S2	*Brevibacillus thermoruber*
EMA22	*Aeribacillus pallidus*
HR12	*Geobacillus thermoleovorans*
HR15	***Aeribacillus pallidus***
1	*Bacillus smithy*
7	*Brevibacillus thermoruber*

Strain BIOF is a Gram-positive bacterium with a shiny, opaque, flat, circular colony. Strain HR15 is also a Gram-positive bacterium with smooth, translucent and irregular colony. Both of them have a range of temperature between 40ºC and 75ºC.

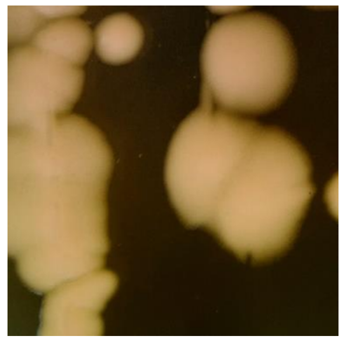

Figure 6: Image of strain BIOF

Figure 7: Image of strain HR15

3.1.1 Cultivation

In 100 mL Erlenmeyer flasks with a fixed column inside as shown in the figure 8, lyophilized strain was inoculated in 60 mL of sterilized bacterial standard medium, also known as BSM, used as a basic cultivation medium. As carbon source, Propionic acid was used and placed inside the column. It was then covered and incubated in 55ºC with agitation of 80 rpm.

The BSM was prepared in the laboratory with 2.0 litters of distillate water and 4.3 g of K_2HPO_4, 3.4 g of KH_2PO_4, 2.0 g of $(NH_4)_2SO_4$, 0.34 g of $MgCl_2.6H_2O$ and 0.1 ml of trace elements containing $ZnSO_4.7H_2O$, 2.5 $g.L^{-1}$, $MnSO_4.H_2O$, 2.5 $g.L^{-1}$ $CuSO_4.5H_2O$, 3.9 $g.L^{-1}$, $FeSO_4.7H_2O$, 2.5 $g.L^{-1}$, $CoCl_2.6H_2O$ 0.09 $g.L^{-1}$, $Na_2B_4O_7.10H_2O$ 0.05 $g.L^{-1}$, $Na_2MoO_4.2H_2O$ 0.05 $g.L^{-1}$. Before used for inoculation, it was first sterilized in Autoclave.

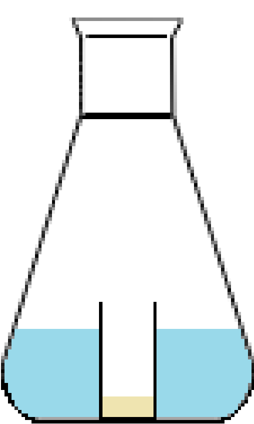

Figure 8: Inoculation of Bacteria.

(The blue color corresponds to the medium where the bacteria was inoculated. The yellow corresponds to the carbon source).

3.2 VOCs and Odorous compounds

For the present work, several carbon sources were chosen as volatile organic compounds: Acetone, α-Pinene, Ethyl acetate, Propionic acid, Toluene, Dimethyl Sulfide and Trimetilamine. As Odorous compounds, some fatty acids were chosen: Butyric Acid, Valeric Acid, Hexanoic Acid.

For the bacterial growth test, α-Pinene, Ethyl acetate, Propionic acid, Toluene and the three odorous compounds were utilized. However for the Bioreactor test, the following VOCs were treated: Acetone, α-Pinene, Ethyl acetate, Propionic acid, Dimethyl Sulfide and Trimetilamine.

The properties of these compounds can be found in annex 2.

3.3 Optical Density Measurement

Optical density measurement of bacterial cultures is a common technique used for measurement of cell density and the growth curve of a pre-determined period. The OD is measured in a spectrophotometer where a visible light is passed through a suspension of cells, and part of it is absorbed and part of it is scattered. The amount of scatter indicates the amount of biomass present in the suspension. The OD of a bacterial culture is thus primarily not an absorbance, as in the case of a dissolved dye. Cells of many bacteria are almost colourless and real light absorption is marginal. It is therefore not correct (even though unfortunately common) to designate the OD of a culture an absorption; the most appropriate term would indeed be turbidity.

The wavelength of OD set in the spectrophotometer depends on growth of the culture. If it is expected a considerable growth, than it is chosen a longer wavelength. If it is expected low growth, the measurement should me more sensitive, being chosen a lower wavelength (Galushko, 2015).

The OD is measured in a spectrophotometer. The underlying principle is that most of the light scattered by the cells no longer reaches the photoelectric cell, so that the electric signal is weaker than with a cell-free cuvette.

- **Growth Curve of Bacteria**

Population growth can be studied by analysing the growth curve of a microbial culture. It usually presents four different phases: Lag, Exponential, Stationary and Death phase

(Willey et al., 2008). Figure 9 represents a typical microbial growth with the districted growth phases.

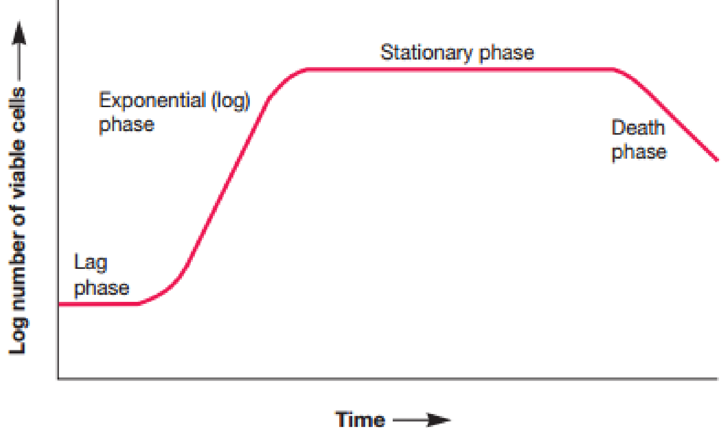

Figure 9: Microbial growth curve (Willey et al., 2008)

The first phase, known as the log phase, is the period in which there is no increment in cell number. It represents the transition period for the bacteria, when transferred to a new condition, to perform various functions such as synthesis of new enzymes to be able to use different nutrients and carbon sources. When conditions are suitable, binary fission begins, entering in the exponential phase. (Spellman, 1999).

The length of log phase varies according to the condition of the microorganisms and the nature of the medium. It may be quite long if the inoculum is prevenient from an old culture or a culture into a chemically different medium. If the inoculum is young and transferred to a fresh medium with the same composition as the previous on, this log phase is either, short or absent(Willey et al., 2008).

The exponential or log phase is the period in which the microorganisms are growing and dividing at the maximal possible rate (Willey et al., 2008). The growth rate is constant and there is a rapid exponential increase in population, doubling regularly until the maximum number of cells is reached. The length of this phase varies, depending on the microorganisms and the composition of the medium in which it is located(Katoh and Yoshida, 2009).

After the exponential phase, the cell growth is limited by the availability of nutrients and accumulation of toxic compounds from the metabolism. It can be also limited by the supply of oxygen. The stationary phase is reached when population growth stops and the growth curve becomes horizontal(Katoh and Yoshida, 2009).

After the stationary phase, cells begin to die and destroy themselves by lysis in the, resulting in a decrease in the cell concentration. This phase in known as declining phase (Katoh and Yoshida, 2009).

3.3.1 Bacterial Growth Test

For the analysis of ability of the cultures to utilize different carbon sources, bacterial growth tests were performed through optical density measurement in microplate reader. The schematics of the solutions distribution on the microplate can be found in the annex 2.

It was carried a two bacterial growth tests for Volatile organic compounds and also two for odorous compounds. The first test was performed for each strain separated, while the second test was done for the mixture of bacteria.

For the first test, each strain was inoculated at 55ºC into 96 plate-wells of EPOCH 2 Microplate reader with with slow intensity shaking of 10 seconds before every reading. Optical density (OD_{450}) was measured every 10 minutes for 22 to 24 hours. For each well, 250 µL of pollutant solution was added along with 30 µL of bacterial suspension, which contained 1 ml of inoculum in 1000 mL of BSM. Blank sample was also made for each pollutant for each concentration. The test was made in duplicate.

The scope of these tests was to understand the degradation behavior of thermophilic bacteria when in contact with the pollutants chosen for this test.

For the second test, different mixtures of the two strains to be used in this work were prepared. The mixture was prepared in three different proportions named P1, P2 and P3.

The proportions were prepared in sterile test tubes. For P1, 7 mL of BSM was added along 2 mL of the inoculum of strain BIOF and 1 mL of strain HR15. P2 was prepared with the same volume of BSM as P1, but for the strain BIOF, 1 mL of its inoculum was added along with 2 mL of strain HR15 inoculum. P3 had a higher volume of BSM in comparison to the other two proportions. It was added 8 ml of it along with 1 mL of each strain inoculum.

The mixture of bacteria was inoculated into the 96 plate-wells at the same way as for the single strain test.

The scope of this test was to understand, when together, if they were able to degrade the pollutants, as well as, to find the best proportion between the strains for the bioreactor.

3.3.1.1 Volatile Organic Compounds

Solutions of each volatile organic compound already mentioned were prepared in different concentration of 10, 50, 100, 200 and 500 $mg.L^{-1}$. The selection of this range of concentrations was based on the odour threshold of these compounds, presented in the annex 2.

3.3.1.2 Odorous Compounds

The same tests were carried out for the odorous compounds already mentioned. Different solutions were prepared in two different concentrations of 50 and 100 $mg.L^{-1}$.

3.4 Evaluation of polyurethane foam as carrier of microbial biofilm

For this study, three types of different biodegradable carrier material were created in cooperation with the Institute of Macromolecular Chemistry in Prague. It is expanded polyurethane (PUR - foams) based on aliphatic polyisocyanate and a polyester polyol containing various starch derivatives. The selected foam will be patent by Dekonta and will be utilized in the Bioreactor for treatment of VOCs and odorous compounds.

The three foams were denominated PurA, PurA 20S and PurA 40S. The table 3 summarizes the properties of the foams.

Table 3: Foam Properties

Name	Specification	ρ [$kg.m^{-3}$]
Pur A	Polyester PUR A foam	70
Pur A 20 S	Polyester PUR foam with 20% weight of acetylate starch	80
Pur A 40 S	Polyester PUR foam with 40% weight of acetylate starch	160

The chemical and physical structure of Pur A foams can be seen in the figure 10 and 11. The physical structure was obtained from images of optical and electron microscopy.

Figure 10: Chemical Structure of the Foam

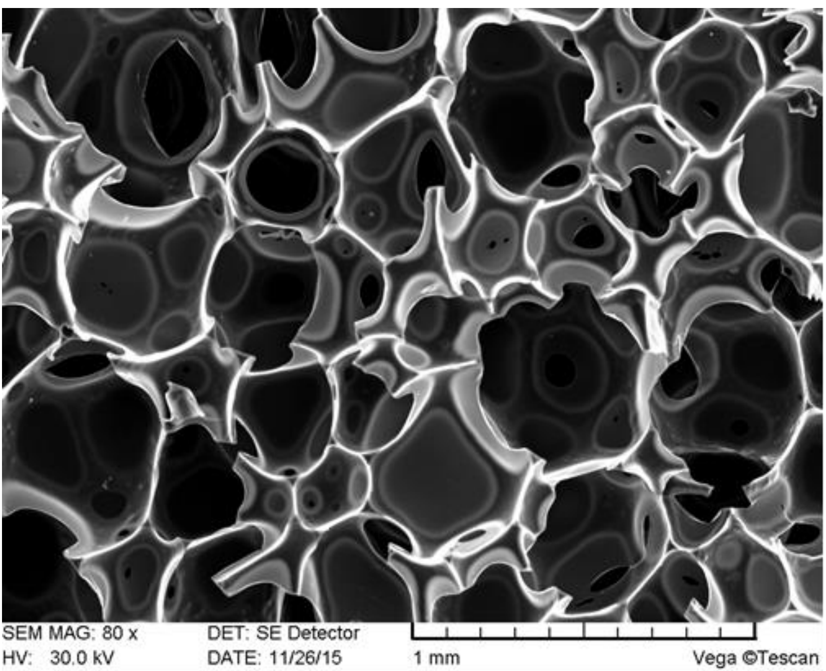

Figure 11: Physical Structure of the Foam

3.4.1 Evaluation of the foam as inoculating material

In biofiltration processes, the inoculation of the biotrickling filter is done by adding the bacteria suspension into the bioreactor. Dekonta, however, wants to analyse if it is possible to use the foam as an inoculating material of the biotrickling filter. For this, a plate counting technique was used.

The test was divided in two parts. The goal of the first part was to quantify the strain colonies when the inoculation is done by adding the bacterial suspension. For the second part, the goal was to quantify the strain colonies when the inoculation is done by the foam.

For the first essay, each foam sample was cut into cubes of 2 grams and dried in 55ºC for one full day. After this procedure, four cubes of each foam were immediately submerged

into 45 mL of Glucose (5 g.L^{-1}) in the Erlenmeyer flasks with the fixed column into where the Carbon source, propionic Acid, was added. In each flask, 2 mL of each bacterial inoculum was added. It was then left for 48 hours in the incubator in 55ºC and 80 rpm rotation.

After the 48 hours, one mL of each suspension in the Erlenmeyer flasks was taken and added into 99 mL of physiological solution. It was then diluted three times and following dilution, 0.1 mL was placed on solid agar for enumeration of total culturable bacteria. They were then incubated for 2 days. The figure 12 describes the dilution procedure.

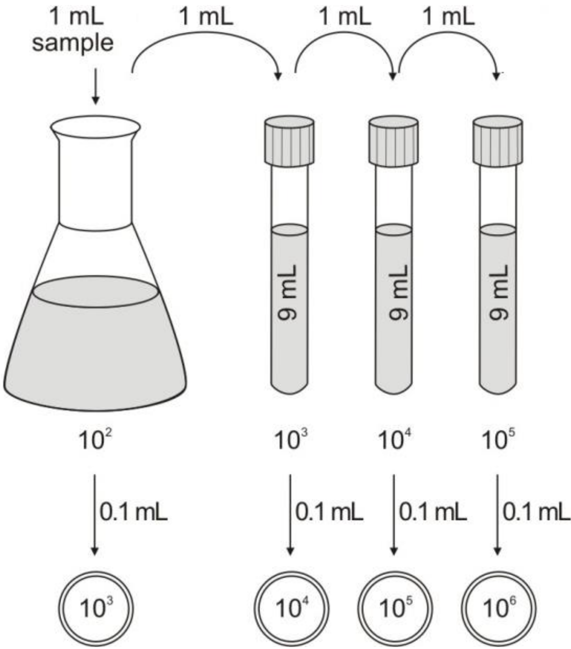

Figure 12: Dilution Procedure

For the second part of this test, the foam cubes found inside the Erlenmeyer flasks from the first part were transferred to new flasks with the same quantity of glucose, but without the addition of inoculum. Then incubate for the same period of time for the previous part in the same conditions, and after the dilution was made for colonies enumeration. If the number of colonies would be the same as the first part of the test, it is possible to conclude that the foam can be used as an inoculating material.

3.4.2 Optical Density Measurement of the Surface Area

The main goal of this test was to determine the capability of the bacteria to create biofilm on the foam samples and which one it was better carrier material for this purpose, through the increase or decrease of the optical density.

In a 6 wells plate, a layer of each type of foam was added into each well and optical density measurement of the surface area was performed.

Immediately after the first test, in each well, 0.9 mL of inoculum of each strain was added, as shown in the figure 13. The optical density was then measured one time and after the plate was incubated in 55ºC. Three more measurements were performed, with a gap of one day between them.

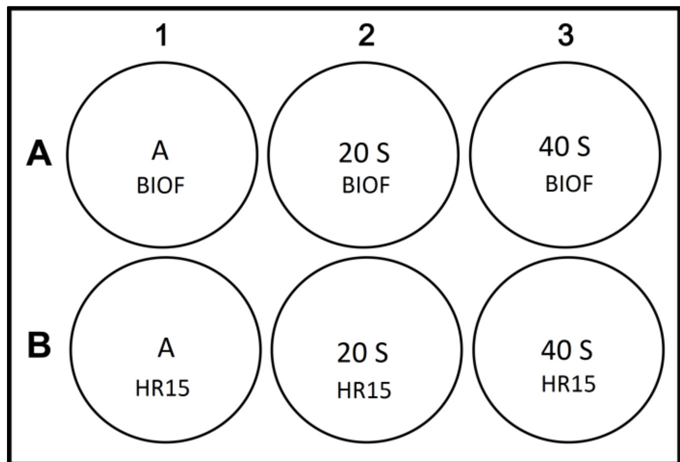

Figure 13: 6-well plate scheme

3.5 Bioreactor Set-UP

The biodegradation experiment was carried out in a laboratory scale Bioreactor filter at the University of Chemistry and Technology, where the selected foam material was used.

It was initially planned to be done in a full scale Biofilter in the laboratory of Dekonta in Slaný, CZ, but due to technical problems, it was not possible to perform the test in this bioreactor.

The bioreactor used for this work was divided into two parts separated by porous wall: bottom part was designed as

The biofilter was inoculated with both strains in BSM and the adaptation in the bioreactors condition lasted 9 days. After the adaptation phase, two tests were performed. The first test, the volatile organic compounds (Acetone, α-Pinene, Ethyl acetate, Propionic acid, Dimethyl Sulfide and Triethylamine) entered the biofilter in a concentration of 660 mg.m^{-3}and in the second test, in a concentration of 1100 mg.m^{-3}. They were dosed into the bioreactor utilizing a Piston Micropumps Gilson 402 syringe pump. Samples were taken

from 2 sampling points located before the inlet and in the outlet and then measured by gas chromatography.

The reactor was heated using the heated bath Sahara series S21P and it was maintained at 55ºC by the insulation cover. The liquid was recirculated continuously in counter current in a ratio of 12 $L.h^{-1}$, utilizing a peristaltic dosing pump.

This experiment is a preliminary test to understand how the bacteria will degrade the volatile organic compounds and calculate the elimination capacity and the removal rate of the pollutants. For lack of time, it was not possible to perform the test for odorous compounds.

The table 4 summarizes the main parameters of the bioreactor.

Table 4: Reactor Characteristics and Standard Operating Conditions

Parameter	Unit	Value
Volume of Trickling Part	L	3
Height of Trickling Part	m	0.44
Volume of Submerged Part	L	3.67
Height of Submerged Part	m	0.42
Total Working Volume	L	6.67
Total Height	m	0.86
Temperature	ºC	55
Packing Bed Surface Area[a]	m^2	210
gas/liquid flow	Counter-Current	
gas flow rate	$L.min^{-1}$	5.10
EBRT[b]	min	1.31
Recycle Liquid Rate	$L.h^{-1}$	12

([a] According to the manufacture, the surface area of 1g of material is around 1m^2; [b]Empty bed retention time = bed volume/gas flow rate)

As shown in the schematics below (figure 14), there is a container of BSM that is pumped into the biofilter every 24 hours in a rate of 1.5 $L.d^{-1}$. This is because along with the outlet air, there is also an outlet of vaporized bioreactor liquid, because of the high temperature, so it is necessary to add an amount of fluid inside the biofilter.

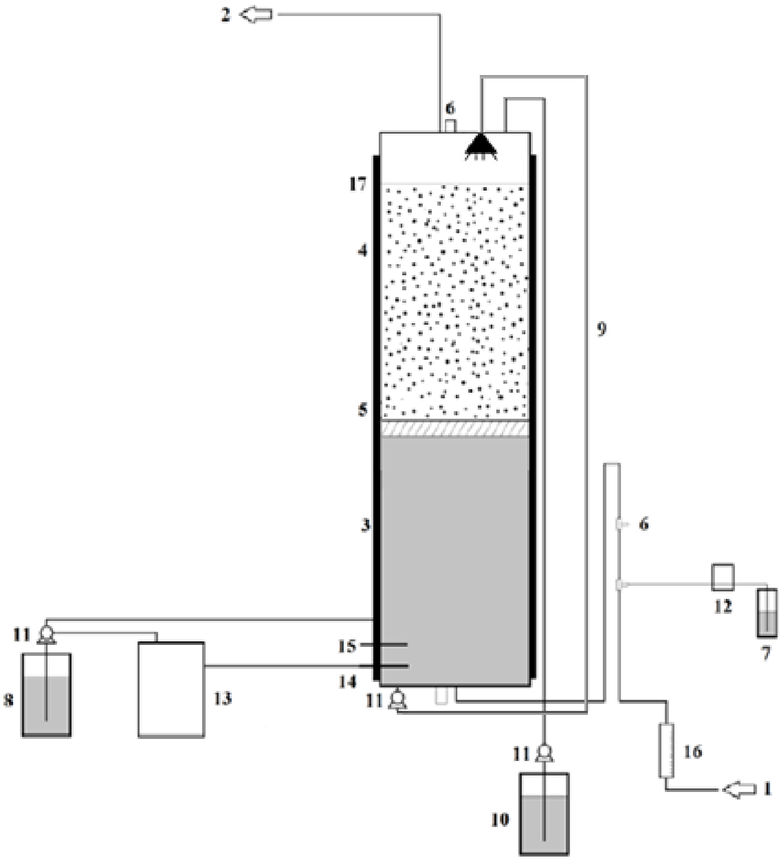

Figure 14: ***Biofilter Set-Up***

(1- Air Inlet; 2- Air Outlet; 3 - Submerged Part; 4- Trickling Part; 5- Porous Wall, 6- Sampling Point, 7 – Carbon Source container; 8- KOH or H2SO4 container; 9- Recirculating Pipe; 10- BSM container; 11- Pump; 12- Piston Pump; 13- pH Regulator; 14 – pH sensor; 15 – Heat Sensor; 16 – Flow Meter; 17 – Insulation cover)

3.5.1 Biofilter's Performance

The description of biofilter's performance is done by two parameters: Elimination Capacity (EC) and Removal Efficiency (RE)

The elimination capacity, EC, provides the amount of pollutant removed per biofilter volume per unit time, and is described by the figure 15.

$$EC = \frac{Q \cdot (C_{g-in} - C_{g-out})}{V} \quad [\mathrm{g\,m^{-3}\,h^{-1}}]$$

Figure 15: Elimination Capacity equation (Waweru et al., 2008)

Removal efficiency, RE, is the fraction of the pollutant removed in the bioreactor expressed as a percentage. It is described by the figure 16:

$$RE = \frac{(C_{\mathrm{g-in}} - C_{\mathrm{g-out}})}{C_{\mathrm{g-in}}} \cdot 100\ [\%]$$

Figure 16: Removal Efficiency equation (Waweru et al., 2008)

Removal efficiency is one of the most used parameters to evaluate the performance of the bioreactor; however, it varies with inlet concentration, airflow and biofilter's volume, and only reflects the specific conditions which is measured. The elimination capacity, on the other hand, allows the comparison between two different biofilter systems, since there is a normalization between the airflow rate and volume (Devinny et al., 1999).

4 Results and Discussion

4.1 Bacterial Growth Test

Both strains were subjected to a bacterial growth tests in order to assess the behaviour of the bacteria when in contact with the pollutants to be studied, through their growth curve. For each strain, it was evaluated the range of concentration in which they grow better. The results will be shown and discussed below.

4.1.1 Bacterial Growth Test for Volatile Organic Compounds

For the assays with separated strains, four graphs were obtained, one of each pollutant (propionic acid, α-Pinene, ethyl acetate and toluene) that will be further discussed. The values of OD in the graphs correspond to the average of the optical density measured for each concentration of each pollutant.

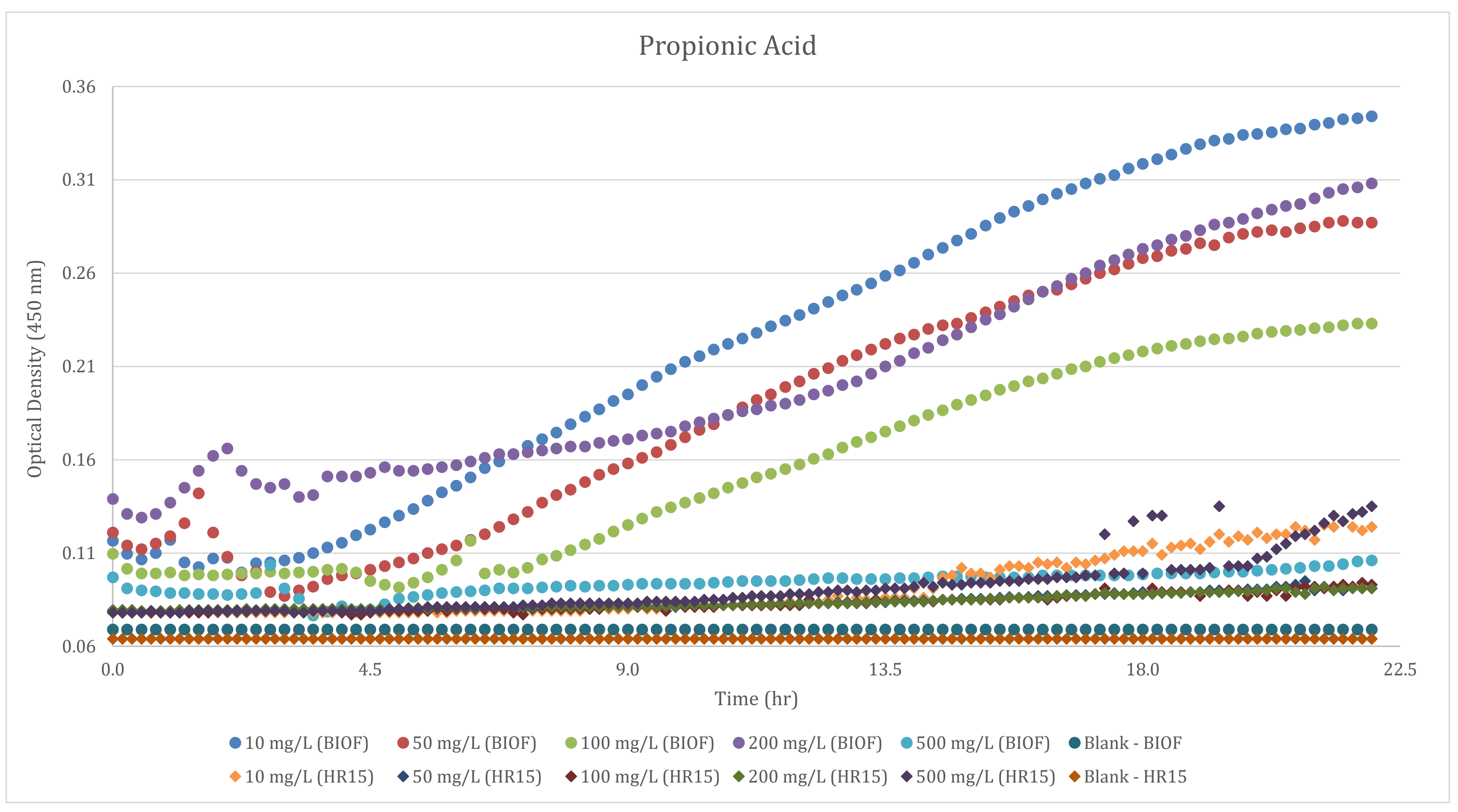

Figure 17: Growth curve of strains BIOF and HR15 in Propionic Acid

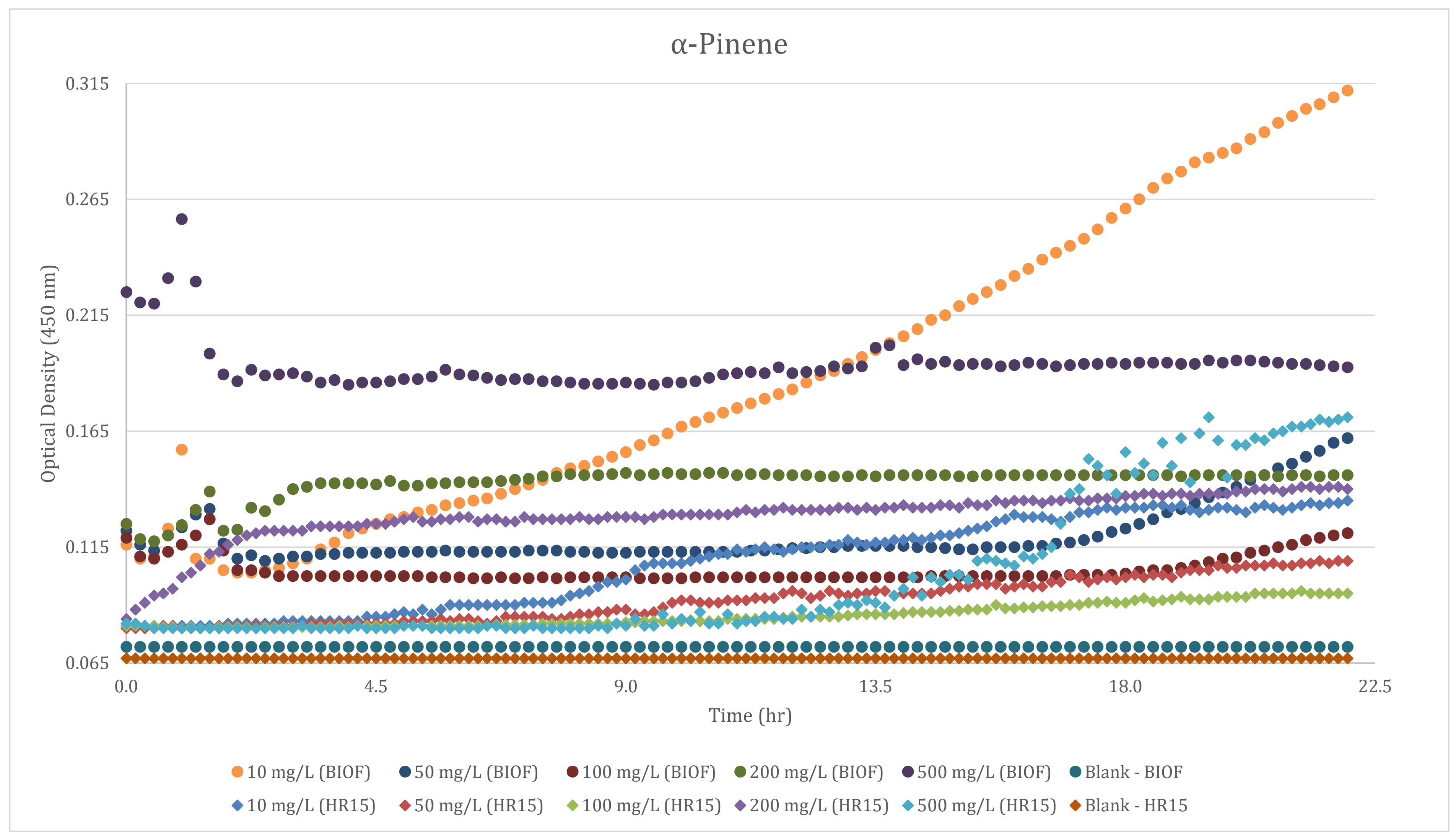

Figure 18: Growth curve of strains BIOF and HR15 in a-Pinene

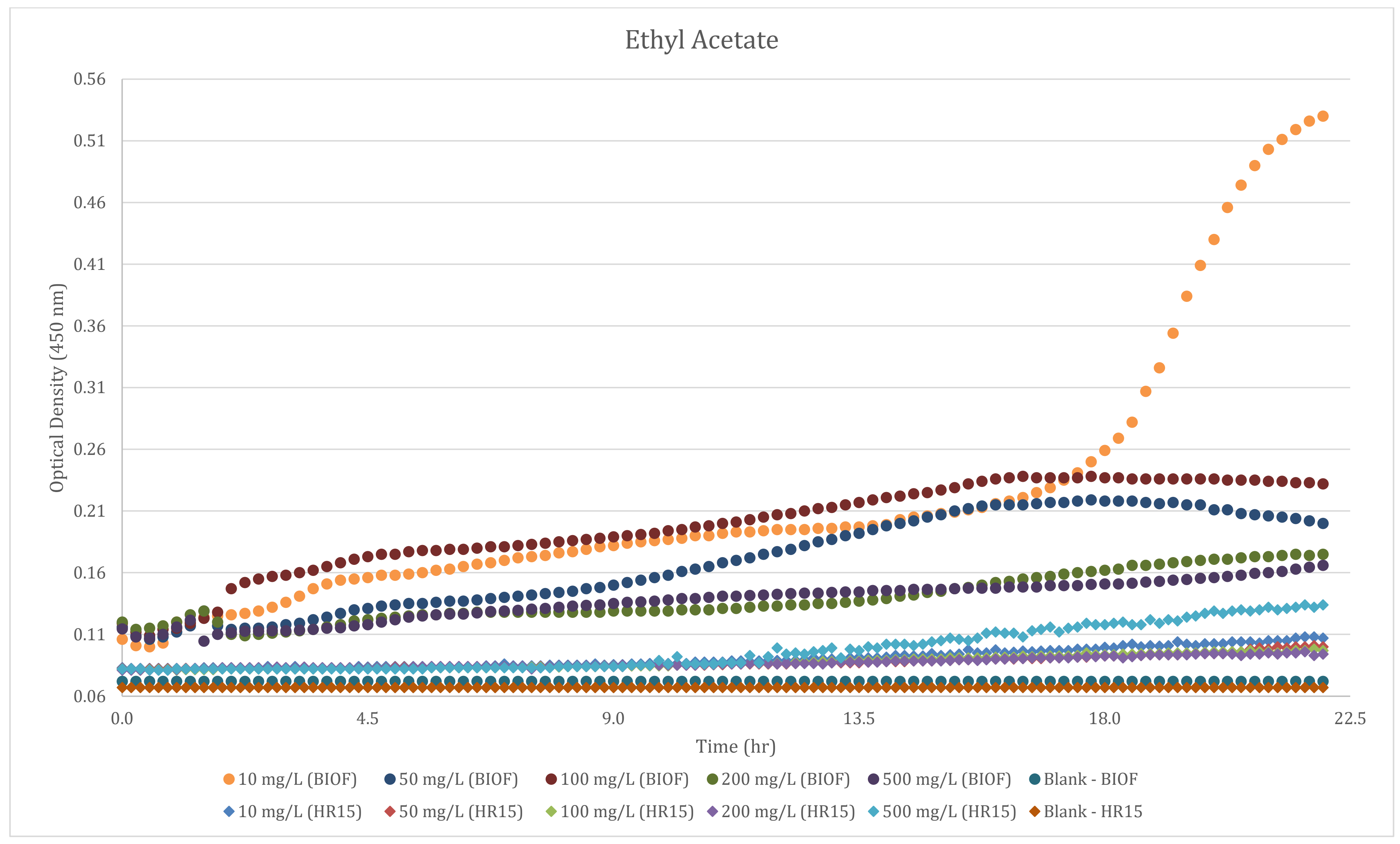

Figure 19: Growth curve of strains BIOF and HR15 in Ethyl Acetate

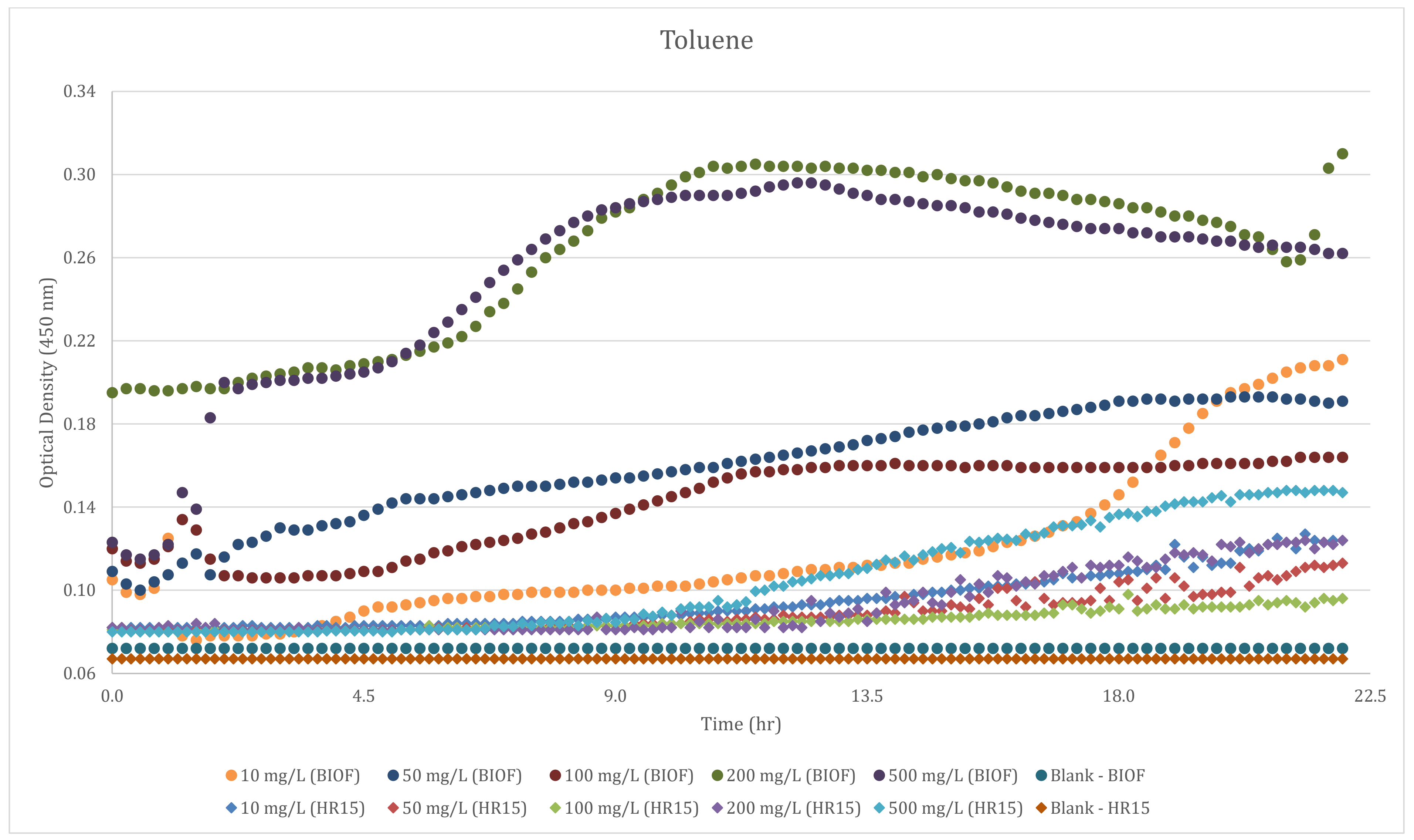

Figure 20: Growth curve of strains BIOF and HR15 in Toluene

When analyzing growth curves obtained by optical density measurement, it is considered growth, when OD increases around three times its value within the test period.

As possible to understand from the graphs, for all the compounds, strain HR15 did not present a considerable growth within the time of the test. For some compounds in some concentrations, it is possible to visualise a beginning of growth, but not enough to evaluate whether it is able to degrade or not the pollutants. A possibility is that this strain may take longer to adapt to the carbon sources.

Strain BIOF, on the contrary showed growth for some of the concentration of all the compounds. For propionic acid, it was able to degrade a concentration range between 10 to 200 $mg.L^{-1}$. The pollutants α-Pinene, Ethyl acetate and toluene were only consumed by the strain in the concentration of 10 $mg.L^{-1}$.

The table 5 presents the specific growth rate of strain BIOF for the pollutants concentration discussed above. It was determine by the slope of the exponential phase of the graph Ln OD x time, presented in the annex 3.

Table 5: Specific Growth Rate (μ) of BIOF strain for the given pollutants

Polutant	Concentration ($mg.L^{-1}$)	μ (h^{-1})
Propionic Acid	10	0.101
	50	0.089
	100	0.069
	200	0.054
α-Pinene	10	0.055
Ethyl Acetate	10	0.027
Toluene	10	0.150

From the table 5, it is possible to observe that the higher the concentration of propionic acid; the lower is the growth rate of strain BIOF. Its rate was decreased by half when the concentration increased from 10 to 200 $mg.L^{-1}$.

Comparing the compounds at a concentration of 10 $mg.L^{-1}$, strain BIOF when utilizing Toluene as carbon source can grow in a much higher rate, when utilizing Ethyl Acetate.

It is possible to conclude that the strain BIOF can degrade both Toluene and Propionic acid in a much higher rate, in comparison to the other pollutants, when their concentration are low.

4.1.1.1 Mixture of Strains

The main goal of this test was to study the degradation behavior of the bacteria when placed together, as well as to analyze the best proportion of strains to be used in the bioreactor test, even though from the previous test, strain HR15 did not show considered growth.

From the previous results, it was able to observe that in higher concentrations, the strains do not degrade the pollutants, therefore for this part of the test concentrations of 10, 50 and 100 mg/L were chosen.

The graphs bellow presents the growth curve for the pollutants in the different proportions.

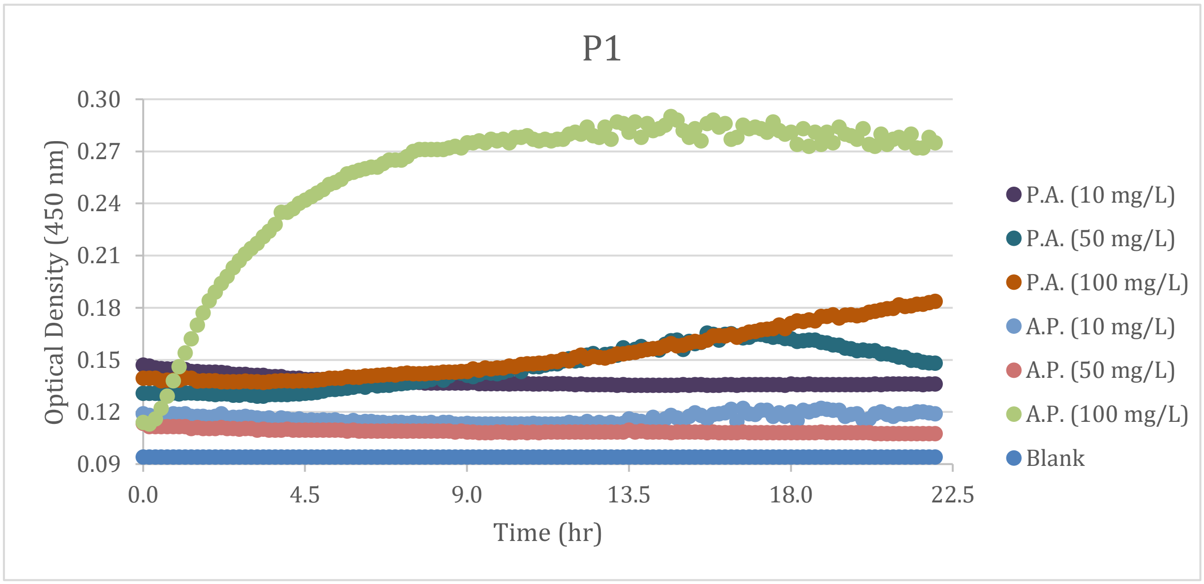

Figure 21: Optical density of the Proportion P1 for Propionic Acid (P.A) and a-Pinene (A.P.)

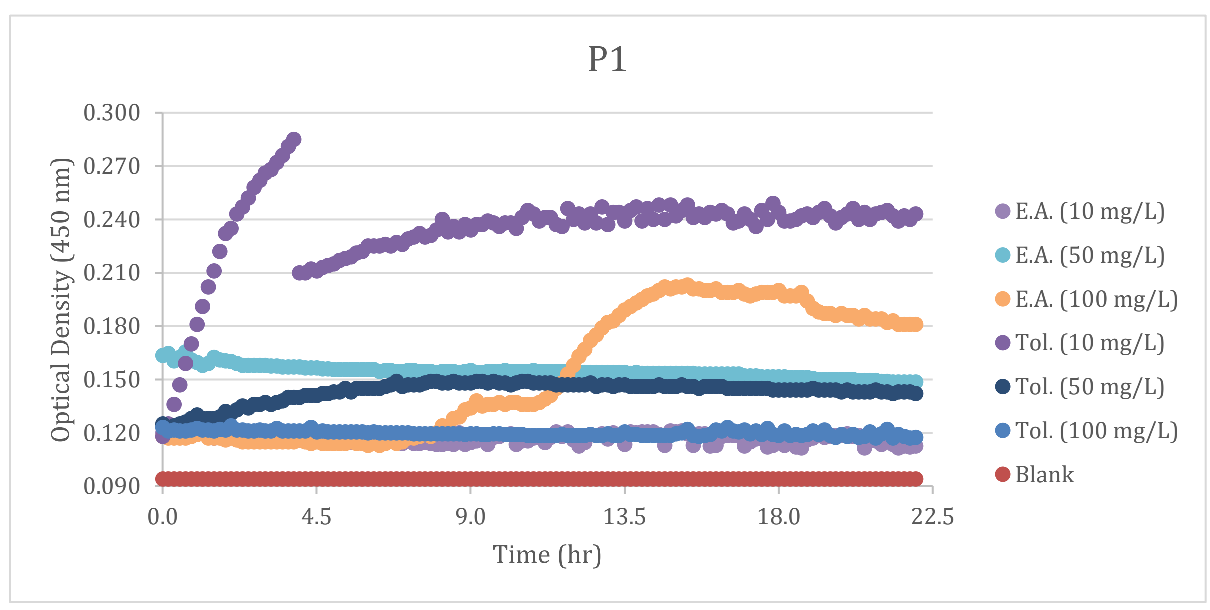

Figure 22: Optical density of the Proportion P1 for Ethyl Acetate (E.A) and Toluene (Tol.)

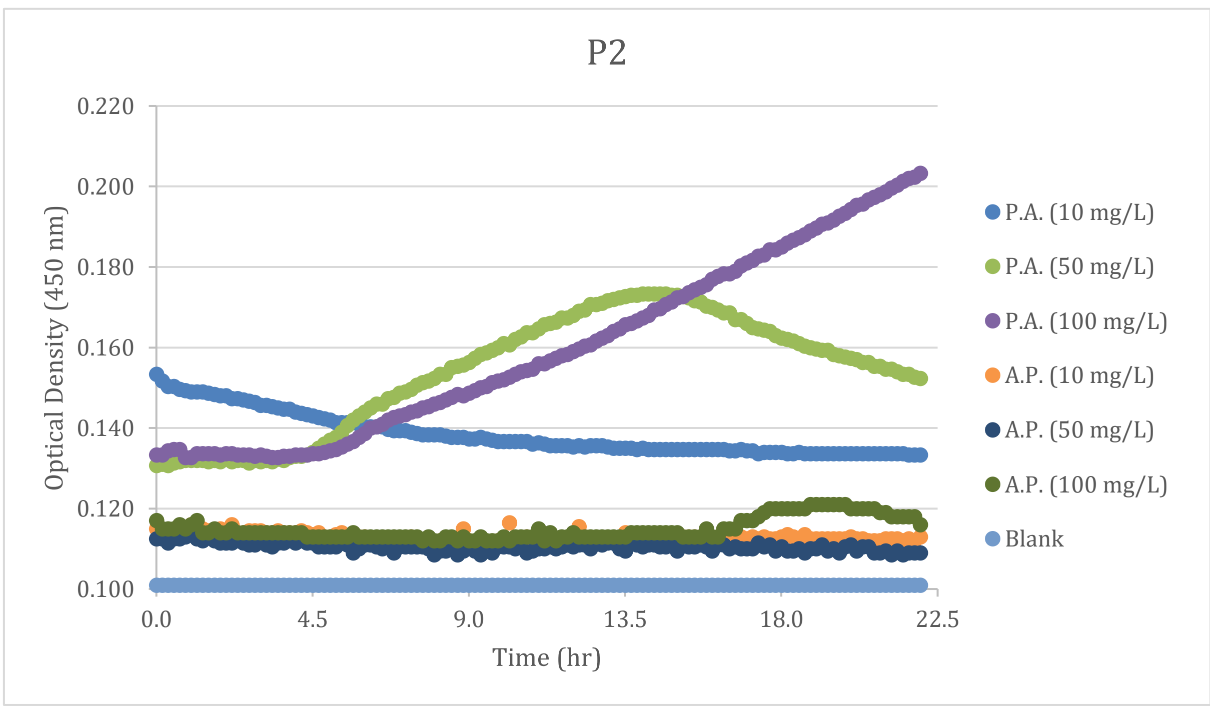

Figure 23: Optical density of the Proportion P2 for Propionic Acid (P.A) and a-Pinene (A.P.)

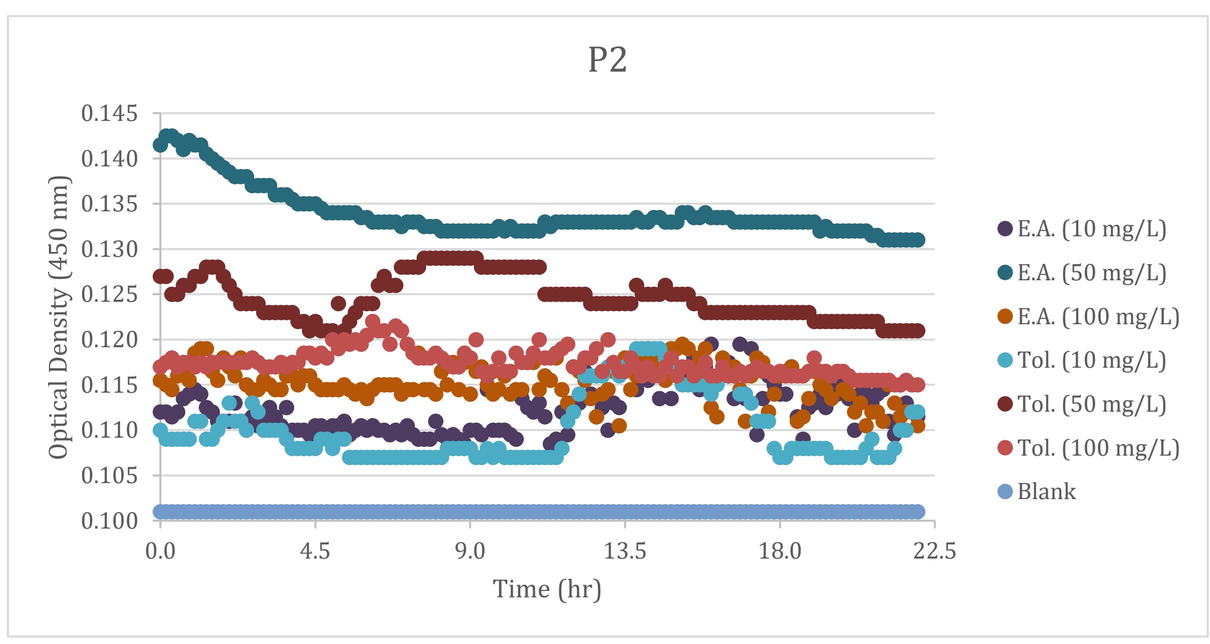

Figure 24: Optical density of the Proportion P2 for Ethyl Acetate (E.A) and Toluene (Tol.)

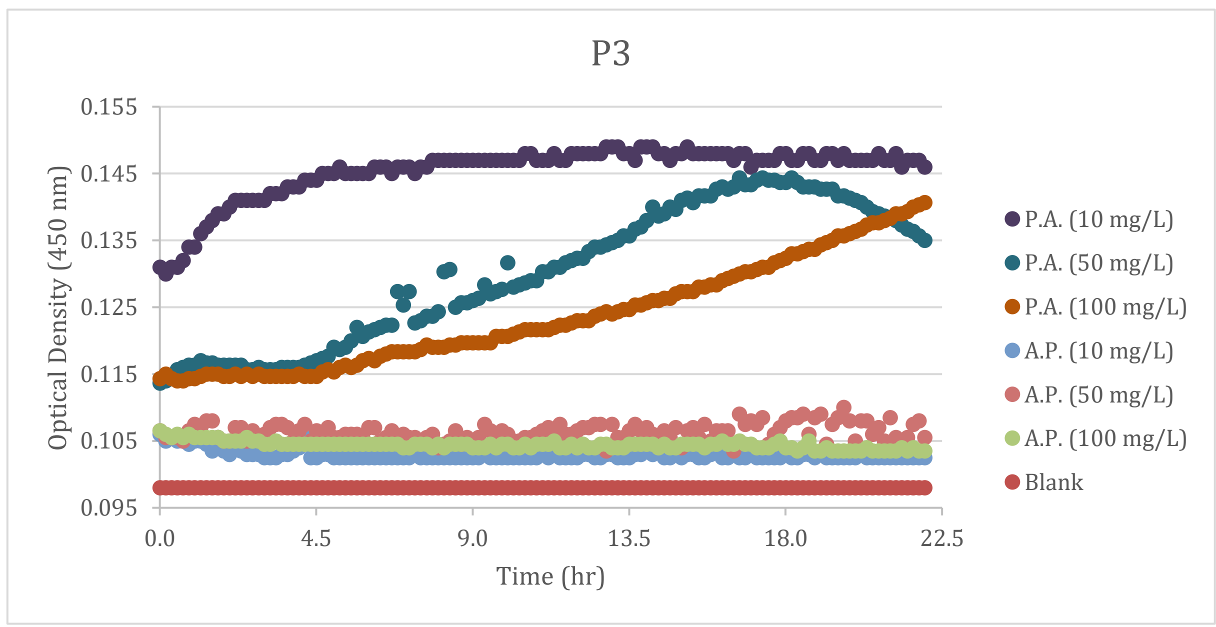

Figure 25: Optical density of the Proportion P3 for Propionic Acid (P.A) and a-Pinene (A.P.)

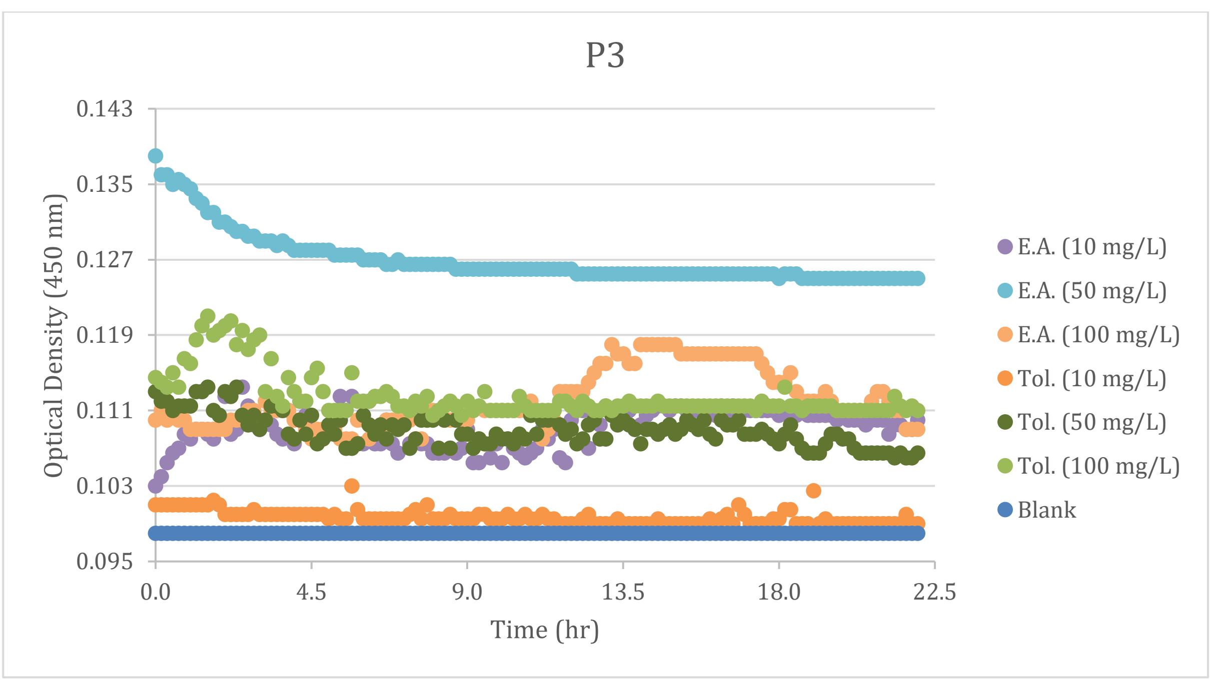

Figure 26: Optical density of the Proportion P3 for Ethyl Acetate (E.A) and Toluene (Tol.)

Such as for the results of the previous tests, the growth curve, in their majority, are not typical growth curve expected. By analyzing the tree graphs, the best option as a proportion was the P1, where the volume of strain BIOF's inoculum is two times bigger than the one for strain HR15. As it can be observed, the curves that show growth has a wider range of exponential phase and presents a better growth in comparison to the other proportions.

4.1.2 Bacterial Growth Test for Odorous compounds

4.1.2.1 Separated Strains

The growth curve for the degradation of the odorous compounds by the strains BIOF and HR15 is represented in the images 25 and 26. For each graph, Ba refers to Butyric Acid, HA to Hexanoic Acid and VA to Valeric Acid.

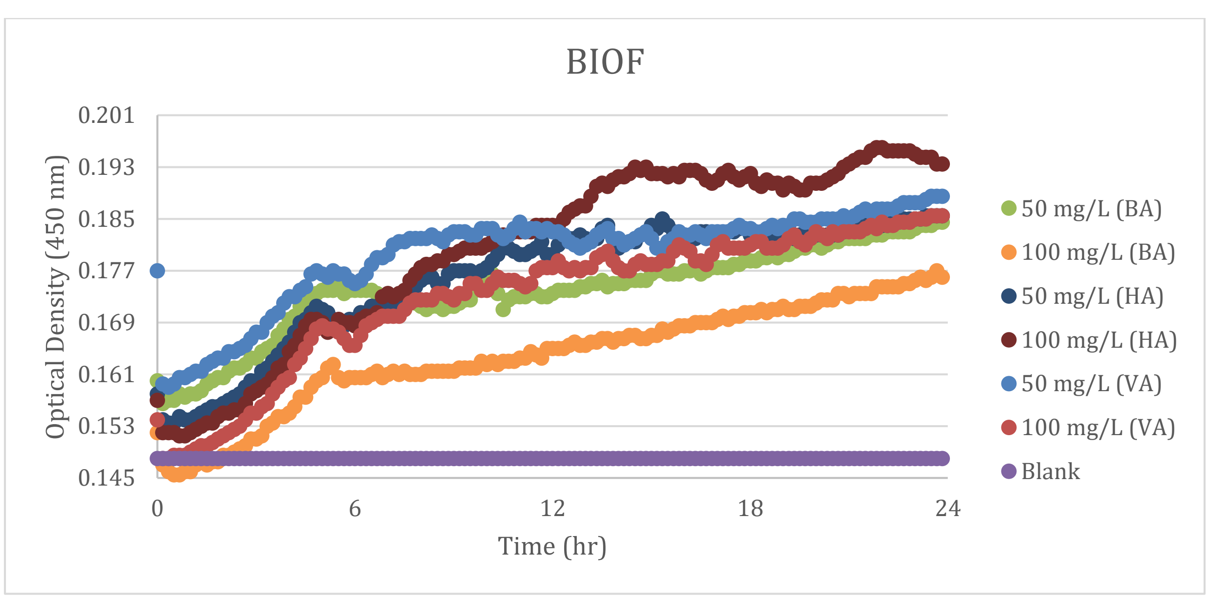

Figure 27: Growth Curve of Strain BIOF for the odorous compounds

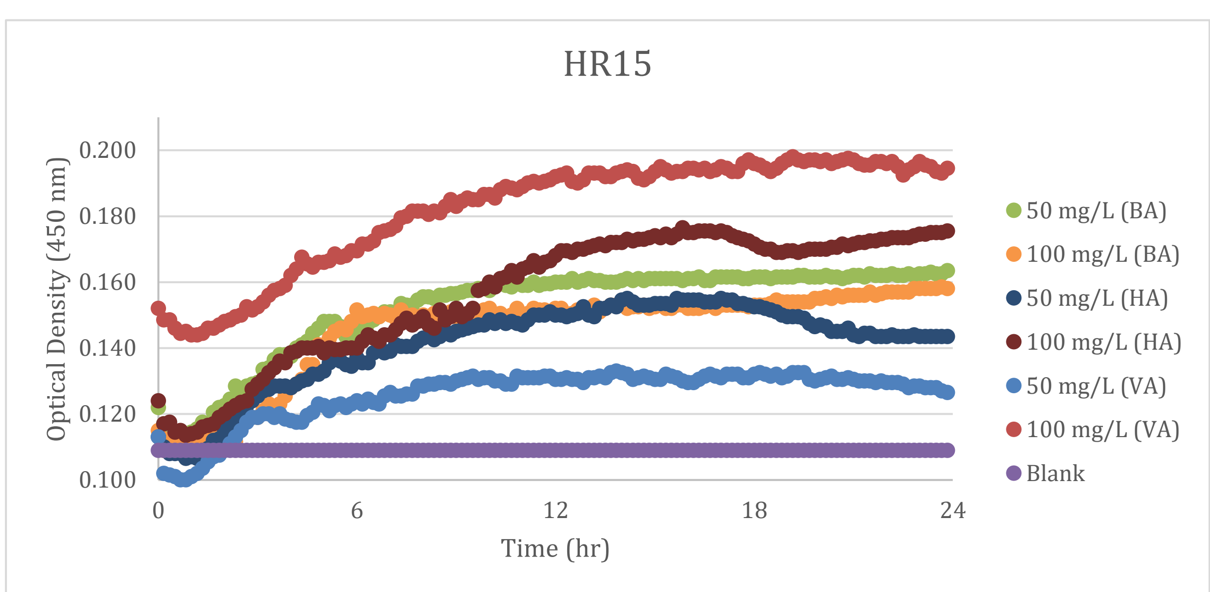

Figure 28: Growth curve of strain HR15 for the odorous compounds

For these compounds, when degraded by strain BIOF, the lag time was almost null, and the exponential phase occurred up to around 6 hours followed by a small decrease of biomass concentration and a linear growth. The stationary phase was not yet observed within the period of the test.

For strain HR15, a small lag phase was observed, followed by a short exponential phase and a stationary phase within 6 to 12 hours depending on the compound and its concentration.

No strain showed a good growth for the odorous compounds in any concentration. It would be necessary a longer test period for strain BIOF to fully evaluate its growth. For strain HR15, test in other concentrations would be necessary to evaluate its range of odorous compounds' biodegradation.

4.1.2.2 Mix of Strains

Such as for volatile organic compounds, a test with the mixture of strain was carried out. The results of each proportion (P1, P2 and P3) are presented in the figures 29, 30 and 31 respectively.

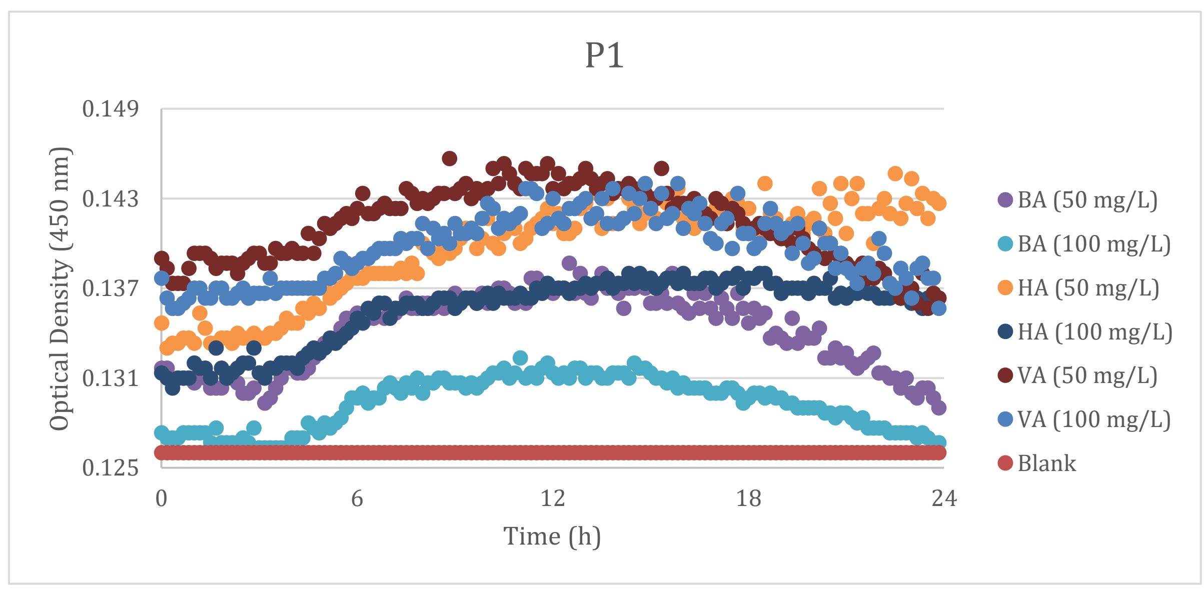

Figure 29: Optical density of the Proportion P1 for the odour compounds

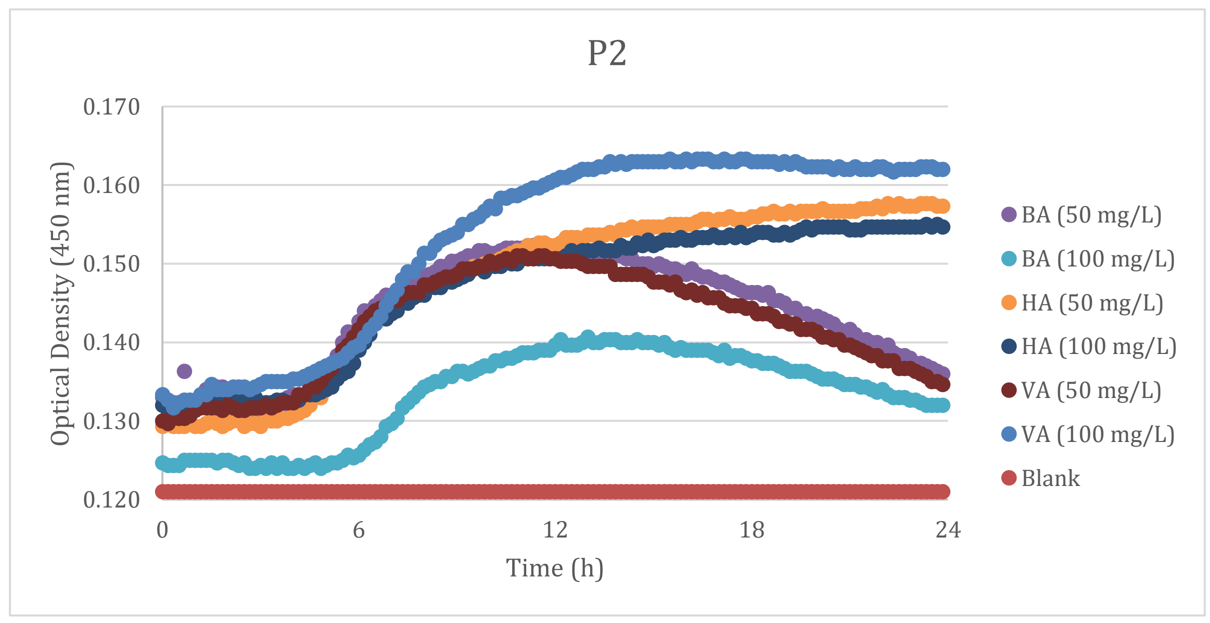

Figure 30: Optical density of the Proportion P2 for the odour compounds

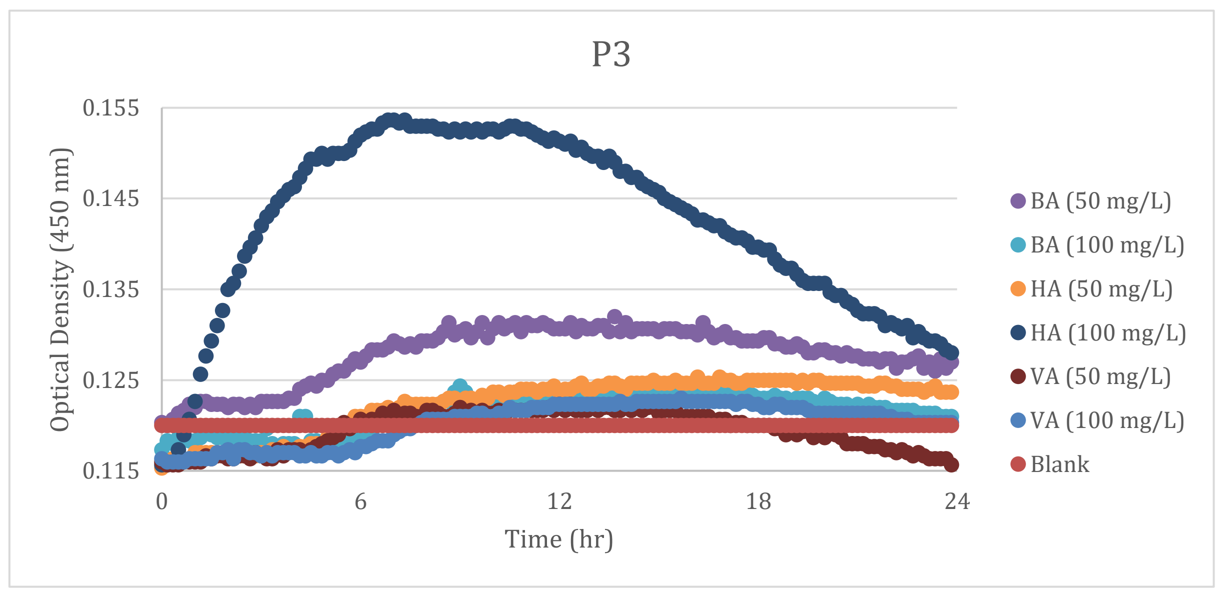

Figure 31: Optical density of the Proportion P3 for the odour compounds

Comparing the three graphs, the best option is the proportion P2 for the treatment of odorous compounds, where the volume of strain HR15 inoculum is two times bigger than the strain BIOF inoculum. For the other proportions, it is observed a small growth quickly followed by stationary and death phase. Although such as for separated strains, another test with different concentrations of these compounds would be necessary to evaluate the best proportion.

4.2 Polyurethane foam as carrier material for the thermophilic biofilter

The next step of the project was to test the ability of the foams as a carrier material of the biofilter and understand if they could be used as an inoculating material. Then determine the best foam to be used in the biofilter.

4.2.1 Evaluation of the foam as inoculating material

After the two days of incubation of the agar plate, it was observed that the colonies were not formed on the agar, but around it, as shown in the figures 32 and 33, making their count impossible.

The cause of this atypical growth around the agar may be explained by the alteration of bacteria cell walls' properties, when interacting with the foam. Glycerol, one of the compounds released from the degradation of the foam, interacts with the cells causing a change in their walls; as result, no colonies were formed.

Since there was no colonies to be counted, the second part of this test was not performed.

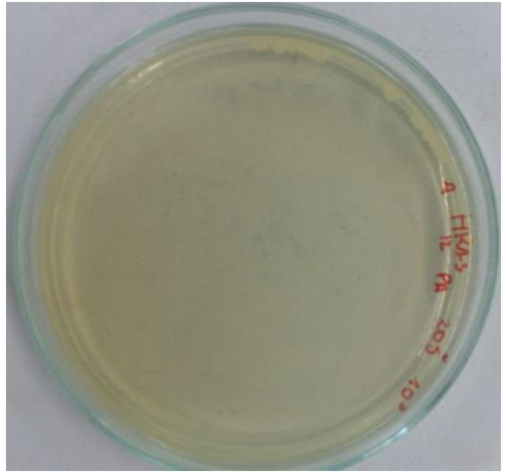

Figure 32: Photo of the agar plate after incubation (top view)

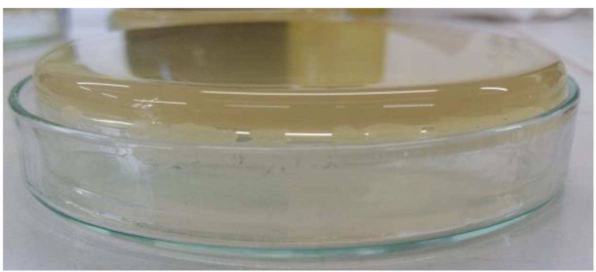

Figure 33: Photo of the agar plate after incubation (front view)

4.2.2 Optical Density measurement of the surface area

The strains BIOF and HR15 were submitted to the optical density measurement test, to study the biofilm formation on the three different foams. The main goal was to compare and identify the best foam for the biofilter through the optical density measurement.

Figure 34 represents the change of the OD in the different measurements for strains BIOF and HR15.

Since it was performed in a 6 well plate, the type of measurement was on the surface area, in other words, several optical density was measured at the same time in different points of the area of one well, and the result was given by the average of the data.

The increase of optical density means the increase of biomass concentration. As can be analysed from the graph, for the foam A+20S and A+40S, the strains were not able to grow and form the biofilm, especially for the higher concentration of starch, where the decrease of biomass was considerable. It is very possible that the strains are sensitive to high concentration of starch.

The foam PUR A was the only material that presented increase of optical density, being the foam chosen as a carrier material for the biofilter.

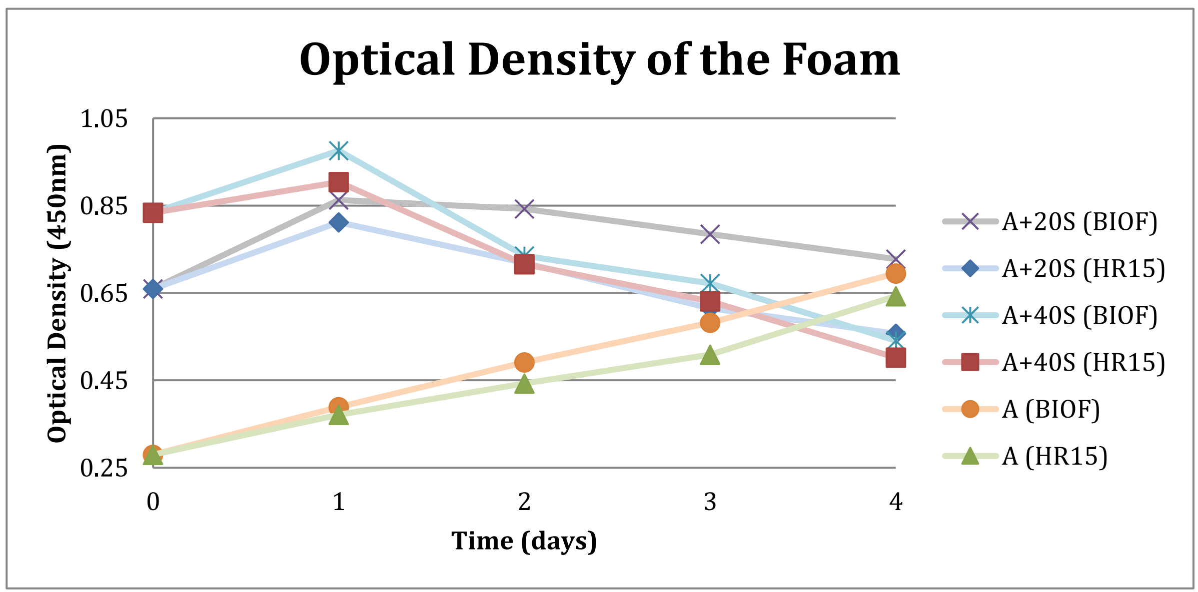

Figure 34: Optical density of Foams Pur A, Pur A 20S and Pur A 40S for BIOF and HR15 strains.

(The measurement 0 corresponds to the OD prior to the inoculum addition)

4.3 Biofiltration Test

Dekonta Company is currently doing a patent on this project; therefore, access to the results of the biofiltration test was limited. The data provided from the company is presented below.

The figure 35 represents the graph, which shows the results of the influence of the pollutants concentration in the elimination capacity of the individual substances at a constant flow, after four hours of treatment.

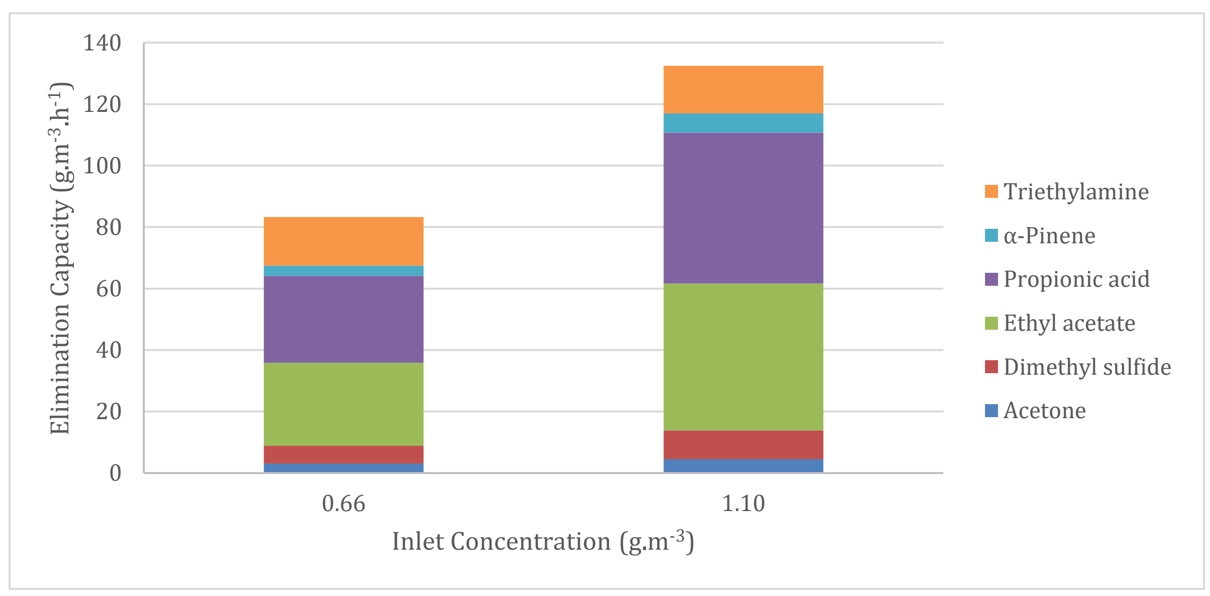

Figure 35: Elimination capacity for selected pollutant with different inlet concentration

The figure 36 represents the influence of the pollutants inlet concentration in the removal efficiency of the individual substances.

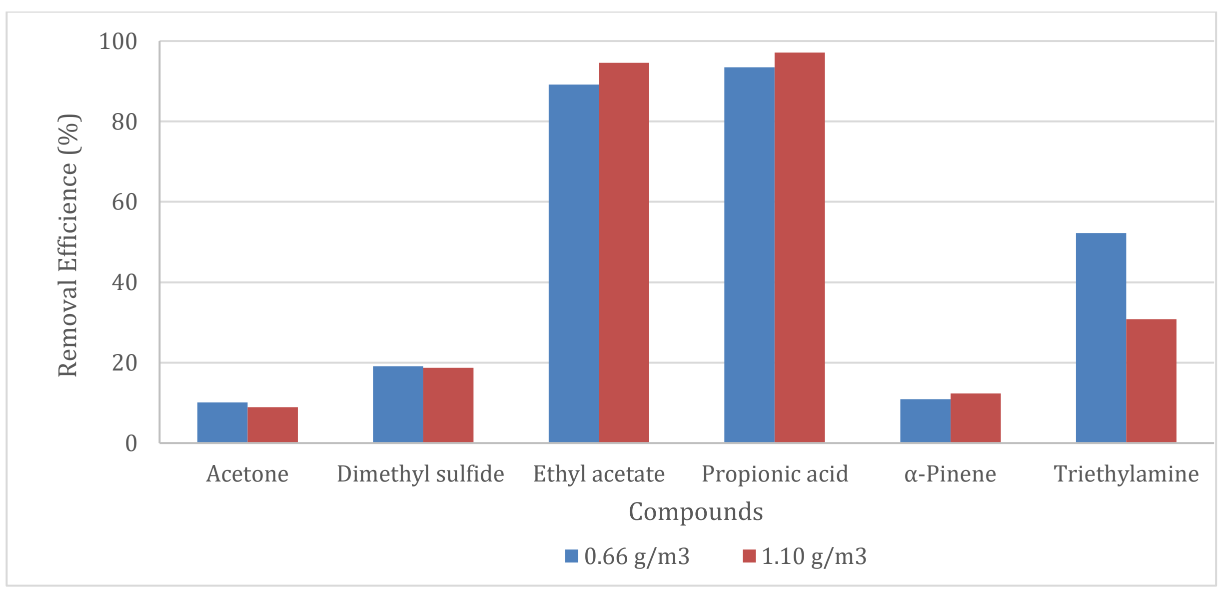

Figure 36: Removal efficiency for selected pollutants in different inlet concentrations

From the graphs, it is possible to verify that propionic acid and ethyl acetate have the higher elimination capacity, being equal to 27.01 $g.m^{-3}.h^{-1}$ and 28.31 $g.m^{-3}.h^{-1}$, respectively, when the inlet concentration was 0.66 $g.m^{-3}$. When the inlet concentration rises to

1.10 $g.m^{-3}$, their elimination capacity increased to 47.73 $g.m^{-3}.h^{-1}$ and 49.02 $g.m^{-3}.h^{-1}$, almost doubling the value. Their removal efficiency is also high, being around 90% in the lower inlet concentration and around 95% in the higher inlet concentration.

The pollutants acetone and α-Pinene were poorly degraded in both tests, having removal efficiency around 10% and elimination capacity, when entering the biofilter with a the lower concentration, equal to 3.06 $g.m^{-3}.h^{-1}$ and 3.31 $g.m^{-3}.h^{-1}$, while for the higher concentration, 4.51 $g.m^{-3}.h^{-1}$ and 6.25 $g.m^{-3}.h^{-1}$. Dimethyl sulfide was slightly better degraded, with removal efficiency around 20% and elimination capacity of 5.80 $g.m^{-3}.h^{-1}$ and 9.44 $g.m^{-3}.h^{-1}$. Triethylamine was the only pollutant that presented a decrease of elimination capacity, even though not so considerable, as well as of removal efficiency, when increasing the inlet concentration.

Therefore, the inlet concentration's variation does not have a significant impact on pollutant's degradation efficiency or elimination capacity, with the exception of ethyl acetate and propionic acid.

Previous studies conducted for the treatment of ethyl acetate in mesophilic biofilter, packed with walnut shells, showed a removal efficiency above 80% when EBRT was equal to 1.25 min (Zare et al., 2012). Biodegradation of propionic acid was evaluated in the bioreactor at mesophilic conditions presented 99% of removal efficiency (Ramírez-Sáenz et al., 2009).

The thermophilic bioreactor utilizing the biodegradable foam at this work was very successful when treating Ethyl Acetate and Propionic Acid with high removal efficiency between 90-97%, showing a similar efficiency to their treatment when in mesophilic conditions. Therefore, the mesophilic biotreatment of these compounds can be successfully replaced by the one presented in this work.

For the other compounds, more tests, with variations of the main parameters (such as inlet concentration, empty bed residence time and inlet flow rate) would be necessary to understand if the thermophilic biofilter packed with the PUR A foam would be able to removal efficiently these pollutants.

5 Conclusions

This study had as main goal the degradation of VOCs in a thermophilic biofilter utilizing a biodegradable carrier material.

Strain BIOF was able to grow with VOCs at low concentrations, of around 10 $mg.L^{-1}$, with the exception of propionic acid, where it grew in a wider higher range (10 to 200 $mg.L^{-1}$). The strain HR15, however, presented a small or no growth within the period of the test. Both strains were not able to present a considerable growth with the tested odorous compounds within the concentration range studied.

Assays were also performed with the mixture of both strains to evaluate their degradation ability when together, as well as, to find the best proportion between the strains. It could be concluded that for the VOCs, the best proportion was when the volume of strain BIOF inoculum was two times higher than the volume of strain HR15. For the odorous compounds, the result was the opposite.

Tests to evaluate the packing material were also conducted. Dekonta selected three types of biodegradable foam to be studied, PUR A, PUR A 20 S and PUR A 40 S, with the aim of evaluating them as an inoculating material as well as to select the best one for the biofiltration. The results showed that the PUR A foam had the best results, however it was not possible to evaluate its capability as a inoculating material.

Finally, results from the thermophilic biofilter utilizing both strains and the selected carrier material showed a successful removal of propionic acid and ethyl acetate, presenting elimination capacity of 27.01 $g.m^{-3}.h^{-1}$ and 28.31 $g.m^{-3}.h^{-1}$, respectively, with removal efficiency of around 90%. Higher elimination capacity of 47.73 $g.m^{-3}.h^{-1}$ and 49.02 $g.m^{-3}.h^{-1}$ respectively was achieved when inlet concentration raised to 1.10 $g.m^{-3}$. At these conditions, their removal efficiency was around 95%. Very similar values of removal efficiency were found in the literature, when treating these compounds at mesophilic conditions.

In conclusion, the replacement of waste treatment in biofilters operated at room conditions for the thermophilic biofilter presented in this work will not only save resources, since there will be no need to cool down the waste gas containing these pollutants, but also maintain the removal efficiency of their treatment.

5.1 Limitations and Future Work

The degradation ability of the pollutants by the strains selected was not possible to be fully evaluated when performing the microplate reader tests. Different ranges of concentrations for both volatile organic compounds and odorous compounds should be studied to obtain the concentration's range in which both strains, especially the strain HR15, are able to degrade without the accumulation of toxic metabolites.

Furthermore, more tests should be performed in the biofilter, to evaluate the influence of gas flow rate as well as of empty bed retention time (EBRT) in the elimination capacity and removal efficiency of the pollutants, therefore to find the best conditions to extend the treatment's efficiency of all the compounds treated.

6 References

Bajpai, P. Biological Odour Treatment, SpringerBriefs in Environmental Science, 2014

Bakke, Rune. BIOFILM DETACHMENT. Doctoral thesis in Civil Engineering - Montana State University, Montana, USA, 1986.

Beloin, Christophe.; Roux; Agnés.; Guigo, Jean-Marc. "Escherichia coli biofilms " Current Topics in Microbiology and Immunology, v. 322, 249-289, Springer-Verlag Berlin Heidelberg (2008).

Brenner-Weiß, G. Bacterial Adhesion: Influence of Conditioning Films (CFs) on the adhesion process. The Research University in the Helmholtz Association (2015)

Chamberlain, A. H. L. The role of adsorbed layers in bacterial adhesion in Biofilms—science and technology, L.F. Melo,T.R. Bott, M. Fletcher, B. Capdeville , 59–67, Kluwer Academic Publishers, (1992).

Characklis, W.G., and K.E. Cooksey. "Biofilms and Microbial Fouling.". Advances in Applied Microbiology, v. 29, 93-138, Academic Press, Inc. (1993)

Cho, K. S., Yoo, S. K., Ryu, H. W. Thermophilic biofiltration of benzene and toluene. Journal of Microbiology and Biotechnology, **17**, 1976-1982. (2007)

Cox, H. H. J., Deshusses, M. A. Biological waste air treatment in biotrickling filters. Current Opinion in Biotechnology, **9**, 256-262 (1998)

Cox, H. H. J., Sexton, T., Shareefdeen, Z. M., Deshusses, M. A. Thermophilic biotrickling filtration of ethanol vapors. *Environmental Science & Technology* **,** *35, (12)*, 2612-2619 (2001)

Cunningham, Alfred, Lennox, John E., and Ross, Rockford J. Biofilms: The Hypertextbook (2008).

Dhamwichukorn, S., Kleinheinz, G.T., Bagley, S.T. Thermophilic biofiltration of methanol and alpha-pinene. Journal of Industrial Microbiology and Biotechnology, **26(3)**, 127-33 (2001)

De Kievit, T. R. and Iglewski, B. H. Quorum sensing, gene expression and Pseudomonas biofilms. Methods in Enzymology, 310, 117-128 (1999)

Delhomenie, M.C., Heitz, M. Biofiltration of Air: A Review. Critical Reviews in Biotechnology, **25**, 53-72 (2005)

Devinny, J. S., Deshusses, M. A., Webster, T. S. Biofiltration for air pollution control. CRC-Lewis, Boca Raton, USA (1999)

Donlan R.M., Biofilms: Microbial life on surfaces. Emerging Infectious Disease journal, 8, 881–890 (2002)

Donlan, R.M., Costerton, J.W. Biofilms: survival mechanisms of clinically relevant Microorganisms. Clinical Microbiology Reviews, 15, 167–193 (2002)

Flemming, H.C., Neu, T.R., Wozniak, D. The EPS matrix: The house of biofilm cells. Journal of Bacteriology, 189, 7945–7947 (2007)

Forsythe, S.J. The microbiology of safe food, 2nd edition, Wiley-Blackwell (2010).

Fulazzaky, M.A., Talaiekhozani, A., Ponraj, M., Majidc, M.Z., Hadibarata, T., Goli, A. Biofiltration process as an ideal approach to remove pollutants from polluted air. Desalination and Water Treatment. 52. 3600-3615 (2014)

Galushko, A. (2015). Why is bacterial density measured at OD 600? Retrieved from https://www.researchgate.net/post/Why_is_bacterial_density_measured_at_OD_600

Govind, R., Narayan, S. Selection of bioreactor media for odor control in Biotechnology for Odor and Air Pollution Control, Z. Shareefdeen, A. Singh, 65-100, Springer Berlin Heidelberg New York (2005)

Hohmann, S., Kögel, S., Brunner, Y., Schmieg, B., Ewald, C., Kirschhöfer, F., Brenner-Weiß, G., Länge, K. Surface Acoustic Wave (SAW) Resonators for Monitoring Conditioning Film Formation. Sensors, **15**, 11873-11888 (2015)

Jabra-Rizk, M.A., Meiller, T.F., James, C.E., Shirtliff, M.E. Effect of farnesol on Staphylococcus aureus biofilm formation and antimicrobial susceptibility. Antimicrobial Agents and Chemotherapy, **50**, 1463-1469 (2006)

Jamal M., Tasneem, U., Hussain, T., Andleeb, S. Bacterial Biofilm: Its Composition, Formation and Role in Human Infections. Journal of Microbiology and Biotechnology, 4(3), 1-13 (2015)

Jeong, G.T., Park, D.H., Lee G.Y., Cha, J.M. Application of two-stage biofilter system for the removal of odorous compounds. Applied Biochemistry and Biotechnology, **132**, 1077–1088 (2006)

Kaplan, J. B. Biofilm dispersal: mechanisms, clinical implications, and potential therapeutic uses. Critical Reviews in Oral Biology & Medicine, 89, 205–218 (2010)

Katoh, S., Yoshida, F. Biochemical Engineering: A Textbook for Engineers, Chemists and Biologists. Wiley-VCH (2009)

Kennes, C., Veiga, M.C. Conventional Biofilters in Bioreactors for waste gas treatment, C. Kennes, M.C. Veiga, 47-98, Kluwer Academic Publishers (2001)

Kong, Z., Farhana, L., Fulthorpe, R., Allen, D.G. Treatment of volatile organic compounds in a biotrickling filter under thermophilic conditions. Environmental Science and Technology, **35**, 4347- 4352 (2001)

Koppmann, R. Volatile organic compounds in the atmosphere, Blackwell Publishing (2007)

Kumar, T.P., Rahul, M., Chandrajit, B. Biofiltration of volatile organic compounds (VOCs) - An overview. Research Journal of Chemical Sciences, **1**, 83–92 (2011)

Lappin-Scott, H.M., Costerton, J.W. Microbial Biofilms. Plant and Microbial Biotechnology Research Series: 5, H. M. Lappin , J. W. Costerton, 15–45, University Press, Cambridge, U.K (2003)

Luvsanjamba, M., Kumar, A., Herman, V.L. Removal of dimethyl sulfide in a thermophilic membrane bioreactor. Journal of Chemical Technology and Biotechnology, **83 (9)**, 1218–1225 (2008)

Luvsanjamba, M., Sercu, B., Kertész, S., Van Langenhove, H. Thermophilic biotrickling filtration of a mixture of isobutyraldehyde and 2-pentanone. Journal of Chemical Technology and Biotechnology, **82**, 74-80 (2007)

Luvsanjamba, M., Sercu, B., Peteghem, J.V., Langenhove, H.V. Long-term operation of a thermophilic biotrickling filter for removal of dimethyl sulphide. Chemical Engineering Journal, 142 (3), 248–255 (2008)

Manuel, C.M.D. *Biofilm dynamics and drinking water stability: Effects of hydrodynamics and surface materials*. Ph.D. thesis, Faculty of Engineering, University of Porto, Porto, Portugal, 2007.

Margesin, R., Schinner, F. Biodegradation and bioremediation of hydrocarbons in extreme environments. Applied Microbiology and Biotechnology, 56 (5-6), 650-663 (2001)

Matteau, Y., Ramsay, B. Active compost biofiltration of toluene. Biodegradation, **8**, 135–41 (1997)

Melo, L. F. Biofilm formation and its role in fixed film processes in The Handbook of Water and Wastewater Microbiology, D. Mara, N. Horan, 337-349, Academic Press, London, UK (2003).

Moe, W. M., Irvine, R.L. Polyurethane Foam Medium For Biofiltration. I: Characterization. Journal of Environmental Engineering, 126(9), 815-825 (2000)

Moe, W. M., Irvine, R.L. Polyurethane Foam Medium For Biofiltration. II: Operation and Performance. Journal of Environmental Engineering , 126(9), 826-832 (2000)

Mudliar, S., Giri, B., Padoley, k., Satpute, D., Dixit, R., Bhatt, P., andey, R., Juwarkar, A., Vaidya, A. Bioreactors for treatment of VOCs and odours—A review, Journal of Environmental Management, **91**, 1039–1054 (2010)

Park, J.S. Biodegradation of Paint VOC Mixtures in Biofilters. PhD Thesis, The University of Texas, Austin, USA, 2004

Plaggemeier, T., Lämmerzahl, O. Treatment of Waste Gas Pollutants in Trickling Filters, in Biotechnology, D. Clark, N. Pazdernik (2nd ed), vol. 11c, 333-344, Wiley-VCH Verlag GmbH, Maastricht-Band (2000)

Rabin, N., Zheng, Z., Opoku-Temeng, C., Du, Z.,Bonsu, E., O Sintim, H. Biofilm formation mechanisms and targets for developing antibiofilm agents. Future Medicine Chemistry, **7(4)**, 493–512 (2015)

Ramírez-Sáenz, D., Zarate-Segura, P.B., Guerrero-Barajas, C., García-Peña, E.I. H_2S and volatile fatty acids elimination by biofiltration: clean-up process for biogas potential use. Journal of Hazardous Materials, 163, 1272-1281 (2009)

Santos, V.P.C.O. Catalytic Oxidation of Volatile Organic Compounds. Tese de doutoramento, Faculdade de Engenharia, Universidade do Porto, Porto, Portugal , 2010

Soccol, C.R., Woiciechowski, A.L., Vandenberghe, S., Soares, M., Neto, G.K., Soccol, V. T. Biofiltration: an emerging technology. Indian Journal Biotechnology, **2**, 396-410 (2003).

Solano, C., Echeverz, M., Lasa, I. Biofilm dispersion and quorum sensing. Current Opinion in Microbiology, 18, 96-104 (2014)

Vankeerbergen, A., Possible approaches towards regulating odoriferous emissions in Characterization and Control of Odours and VOC in thc Process Industries, S. Vigneron, J. Hermia, J. Chaouki, Elscvier Science (1994)

Willey, J. M., Sherwood, L., Woolverton, C. J., Prescott, L. M. Prescott, Harley, and Klein's microbiology. McGraw-Hill Higher Education (2008)

Waweru, M., Herrygers, V. , Van Langenhove,H., Verstraete, W. Process Engineering of Biological Waste Gas Purification in Biotechnology Set, 2nd edition, H.-J. Rehm, G. Reed, 260-272, Wiley Online Library (2008).

Zare, H., Najafpour, G., Rahimnejad, M, , Tardast, A., Gilani, S. Biofiltration of ethyl acetate by Pseudomonas putida immobilized on walnut shell. Bioresource Technology, 123, 419-423 (2012)

7 Annexes

Annex 1 – Compound's Properties

Annex 2 – Distribution of solutions in the 96 plate-well of Microplate Reader

Annex 3 – Specific Growth Rate Determination

Annex 4 – Laboratory-Scale Bioreactor

Annex 5 – Biofilm formation on the Carrier Material

Annex 1. Compounds' Properties

Compound	Purity (%)	Molecular Formula	Molecular Weight ($g.mol^{-1}$)	Structure	Odour Threshold		Odour
					ppm	$mg.m^{-3}$	
Acetone	99.9	C_3H_6O	58.08		100	237.54	Fruity odour
Butyric Acid	99.0	$C_4H_8O_2$	88.11		10^{-3}	$3.60x10^{-3}$	rancid odour
Dimethyl Sulfide	98.0	C_2H_6S	62.13		10^{-3}	$2.54x10^{-3}$	wild radish, cabbage-like odour
Ethyl Acetate	99.7	$C_4H_8O_2$	88.11		$5x10^{-3}$	$1.80x10^{-2}$	ether-like odour, reminiscent of pineapple
Hexanoic Acid	99,0	$C_6H_{12}O_2$	116.16		$6x10^{-3}$	$2.85x10^{-2}$	goat-like odour
α- Pinene	98.0	$C_{10}H_{16}$	136.23		$6x10^{-3}$	$3.34x10^{-2}$	odour of pine

Propionic Acid	99.0	$C_3H_6O_2$	74.08		-	0.084	sour
Toluene	99.0	C_7H_8	92.14		2.14	8.06	sweet, pungent, benzene-like odour
Triethylamine	99.5	$C_6H_{15}N$	101.19		$0.54x10^{-2}$	$2.24x10^{-2}$	strong, ammonia-like odour
Valeric Acid	99.0	$C_5H_{10}O_2$	102.13		$0.37\ x10^{-4}$	$0.15\ x10^{-3}$	similar to butyric acid

Annex 2. Distribution of solutions in the 96 plate-well of Microplate Reader

Table 6: Microplate scheme of separated strains in volatile organic compounds

	1	2	3	4	5	6	7	8	9	10	11	12
A	Propionic Acid [10]	Propionic Acid [10]	Bl	Propionic Acid [50]	Propionic Acid [50]	Bl	Propionic Acid [100]	Propionic Acid [100]	Bl	Propionic Acid [200]	Propionic Acid [200]	Bl
B	Propionic Acid [500]	Propionic Acid [500]	Bl	α-Pinene [10]	α-Pinene [10]	Bl	α-Pinene [50]	α-Pinene [50]	Bl	α-Pinene [100]	α-Pinene [100]	Bl
C	α-Pinene [200]	α-Pinene [200]	Bl	α-Pinene [500]	α-Pinene [500]	Bl	Ethyl Acetate [10]	Ethyl Acetate [10]	Bl	Ethyl Acetate [50]	Ethyl Acetate [50]	Bl
D	Ethyl Acetate [100]	Ethyl Acetate [100]	Bl	Ethyl Acetate [200]	Ethyl Acetate [200]	Bl	Ethyl Acetate [500]	Ethyl Acetate [500]	Bl	Toluene [10]	Toluene [10]	Bl
E	Toluene [50]	Toluene [50]	Bl	Toluene [100]	Toluene [100]	Bl	Toluene [200]	Toluene [200]	Bl	Toluene [500]	Toluene [500]	Bl
F	x	x	x	x	x	x	x	x	x	x	x	x
G	x	x	x	x	x	x	x	x	x	x	x	x
H	x	x	x	x	x	x	x	x	x	x	x	x

(Bl corresponds to the blank samples; 'x' to empty well, number inside the parentheses corresponds to the concentration of pollutant in $mg.L^{-1}$)

Table 7: Microplate scheme for separated strains in odorous compounds

	1	2	3	4	5	6	7	8	9	10	11	12
A	Butyric Acid [50]	Butyric Acid [50]	Bl	Butyric Acid [100]	Butyric Acid [100]	Bl	Hexanoic Acid [50]	Hexanoic Acid [50]	Bl	Hexanoic Acid [100]	Hexanoic Acid [100]	Bl
B	Valeric Acid [50]	Valeric Acid [50]	Bl	Valeric Acid [100]	Valeric Acid [100]	Bl	x	x		x	x	x
C	x	x	x	x	x	x	x	x	x	x	x	x
D	Butyric Acid [50]	Butyric Acid [50]	Bl	Butyric Acid [100]	Butyric Acid [100]	Bl	Hexanoic Acid [50]	Hexanoic Acid [50]	Bl	Hexanoic Acid [100]	Hexanoic Acid [100]	Bl
E	Valeric Acid [50]	Valeric Acid [50]	Bl	Valeric Acid [100]	Valeric Acid [100]	Bl	x	x		x	x	x
F	x	x	x	x	x	x	x	x	x	x	x	x
G	x	x	x	x	x	x	x	x	x	x	x	x
H	x	x	x	x	x	x	x	x	x	x	x	x

(For the wells located in the lines A and B, the strain BIOF was utilized, for the wells located in the lines D and E, strain HR15 was used)

Table 8: Microplate scheme of the strains mixture in propionic acid and α-pinene

	1	2	3	4	5	6	7	8	9	10	11	12
A	Propionic Acid P1 [10]	Propionic Acid P1 [10]	Bl	Propionic Acid P2 [10]	Propionic Acid P2 [10]	Bl	Propionic Acid P3 [10]	Propionic Acid P3 [10]	Bl	Propionic Acid P1 [50]	Propionic Acid P1 [50]	Bl
B	Propionic Acid P2 [50]	Propionic Acid P2 [50]	Bl	Propionic Acid P3 [50]	Propionic Acid P3 [50]	Bl	Propionic Acid P1 [100]	Propionic Acid P1 [100]	Bl	Propionic Acid P2 [100]	Propionic Acid P2 [100]	Bl
C	Propionic Acid P3 [100]	Propionic Acid P3 [100]	Bl	α-Pinene P1 [10]	α-Pinene P1 [10]	Bl	α-Pinene P2 [10]	α-Pinene P2 [10]	Bl	α-Pinene P3 [10]	α-Pinene P3 [10]	Bl
D	α-Pinene P1 [50]	α-Pinene P1 [50]	Bl	α-Pinene P2 [50]	α-Pinene P2 [50]	Bl	α-Pinene P3 [50]	α-Pinene P3 [50]	Bl	α-Pinene P1 [100]	α-Pinene P1 [100]	Bl
E	α-Pinene P2 [100]	α-Pinene P2 [100]	Bl	α-Pinene P3 [100]	α-Pinene P3 [100]	Bl	x	x	x	x	x	x
F	x	x	x	x	x	x	x	x	x	x	x	x
G	x	x	x	x	x	x	x	x	x	x	x	x
H	x	x	x	x	x	x	x	x	x	x	x	x

Table 9: Microplate scheme of the strains mixture in ethyl acetate and toluene

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ethyl Acetate P1 [10]	Ethyl Acetate P1 [10]	Bl	Ethyl Acetate P2 [10]	Ethyl Acetate P2 [10]	Bl	Ethyl Acetate P3 [10]	Ethyl Acetate P3 [10]	Bl	Ethyl Acetate P1 [50]	Ethyl Acetate P1 [50]	Bl
B	Ethyl Acetate P2 [50]	Ethyl Acetate P2 [50]	Bl	Ethyl Acetate P3 [50]	Ethyl Acetate P3 [50]	Bl	Ethyl Acetate P1 [100]	Ethyl Acetate P1 [100]	Bl	Ethyl Acetate P2 [100]	Ethyl Acetate P2 [100]	Bl
C	Ethyl Acetate P3 [100]	Ethyl Acetate P3 [100]	Bl	Toluene P1 [10]	Toluene P1 [10]	Bl	Toluene P2 [10]	Toluene P2 [10]	Bl	Toluene P3 [10]	Toluene P3 [10]	Bl
D	Toluene P1 [50]	Toluene P1 [50]	Bl	Toluene P2 [50]	Toluene P2 [50]	Bl	Toluene P3 [50]	Toluene P3 [50]	Bl	Toluene P1 [100]	Toluene P1 [100]	Bl
E	Toluene P2 [100]	Toluene P2 [100]	Bl	Toluene P3 [100]	Toluene P3 [100]	Bl	x	x	x	x	x	x
F	x	x	x	x	x	x	x	x	x	x	x	x
G	x	x	x	x	x	x	x	x	x	x	x	x
H	x	x	x	x	x	x	x	x	x	x	x	x

Table 10: Microplate scheme of the strains mixture in odorous compounds

	1	2	3	4	5	6	7	8	9	10	11	12
A	Butyric Acid P1 [50]	Butyric Acid P1 [50]	Bl	Butyric Acid P2 [50]	Butyric Acid P2 [50]	Bl	Butyric Acid C [50]	Butyric Acid C [50]	Bl	Butyric Acid P1 [100]	Butyric Acid P1 [100]	Bl
B	Butyric Acid P2 [100]	Butyric Acid P2 [100]	Bl	Butyric Acid C [100]	Butyric Acid C [100]	Bl	Hexanoic Acid P1 [50]	Hexanoic Acid P1 [50]	Bl	Hexanoic Acid P2 [50]	Hexanoic Acid P2 [50]	Bl
C	Hexanoic Acid C [50]	Hexanoic Acid C [50]	Bl	Hexanoic Acid P1 [100]	Hexanoic Acid P1 [100]	Bl	Hexanoic Acid P2 [100]	Hexanoic Acid P2 [100]	Bl	Hexanoic Acid P3 [100]	Hexanoic Acid P3 [100]	Bl
D	Valeric Acid P1 [50]	Valeric Acid P1 [50]	Bl	Valeric Acid P2 [50]	Valeric Acid P2 [50]	Bl	Valeric Acid C [50]	Valeric Acid C [50]	Bl	Valeric Acid P1 [100]	Valeric Acid P1 [100]	Bl
E	Valeric Acid P2 [100]	Valeric Acid P2 [100]	Bl	Valeric Acid C [100]	Valeric Acid C [100]	Bl	x	x	x	x	x	x
F	x	x	X	x	x	x	x	x	x	x	x	x
G	x	x	X	x	x	x	x	x	x	x	x	x
H	x	x	X	x	x	x	x	x	x	x	x	x

Annex 3. Specific Growth Rate (μ) Determination

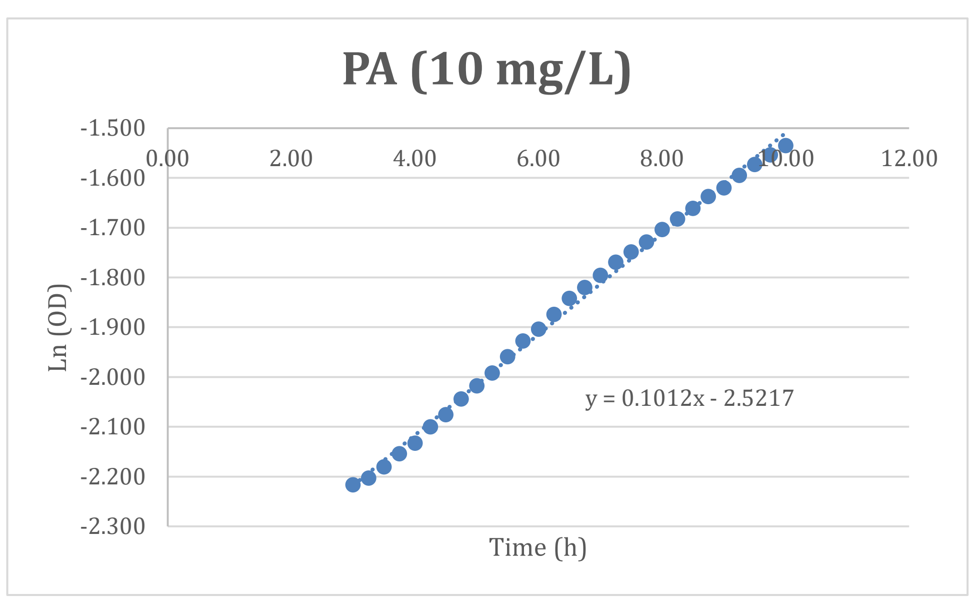

Figure 37**: determination of μ for Propionic acid (10 mg/L)**

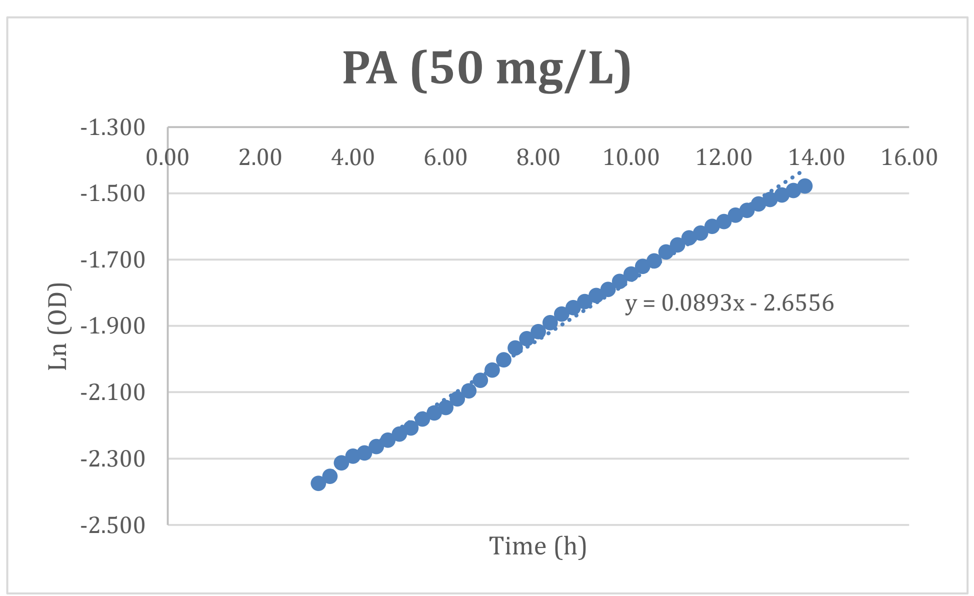

Figure 38: ***determination of μ for Propionic acid (50 mg/L)***

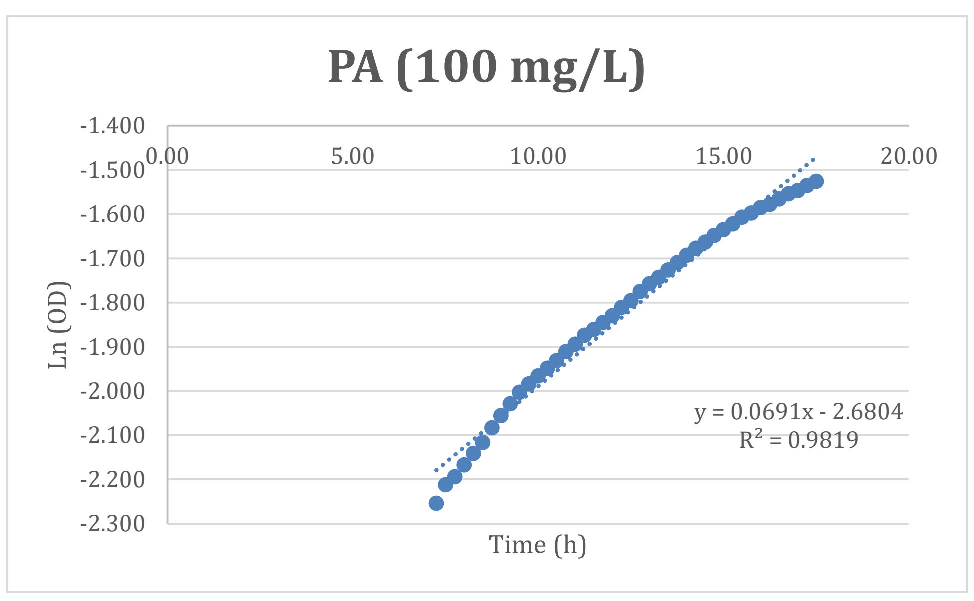

Figure 39: ***determination of μ for Propionic acid (100 mg/L)***

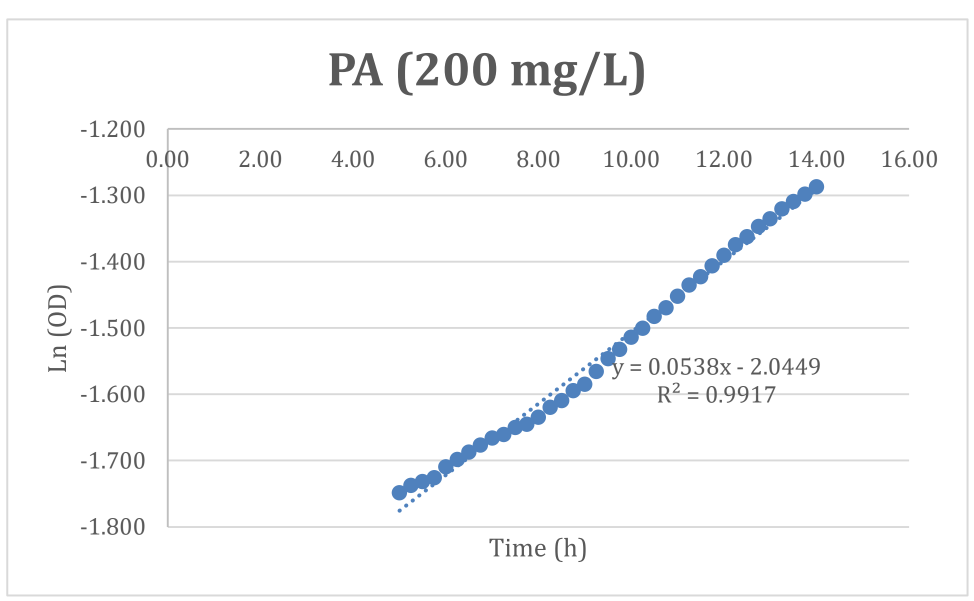

Figure 40: ***determination of μ for Propionic acid (200 mg/L)***

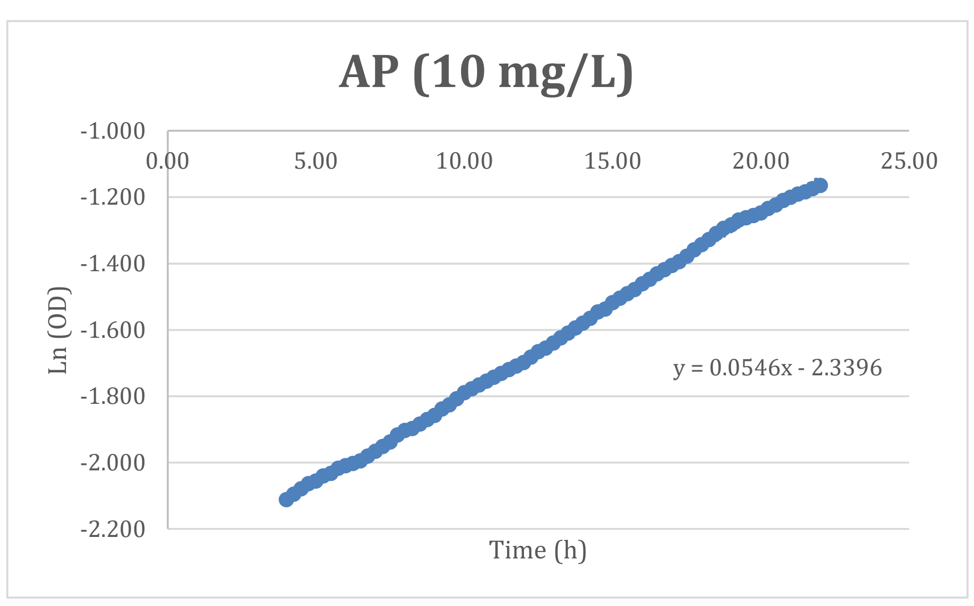

Figure 41: ***determination of μ for a-Pinene (10 mg/L)***

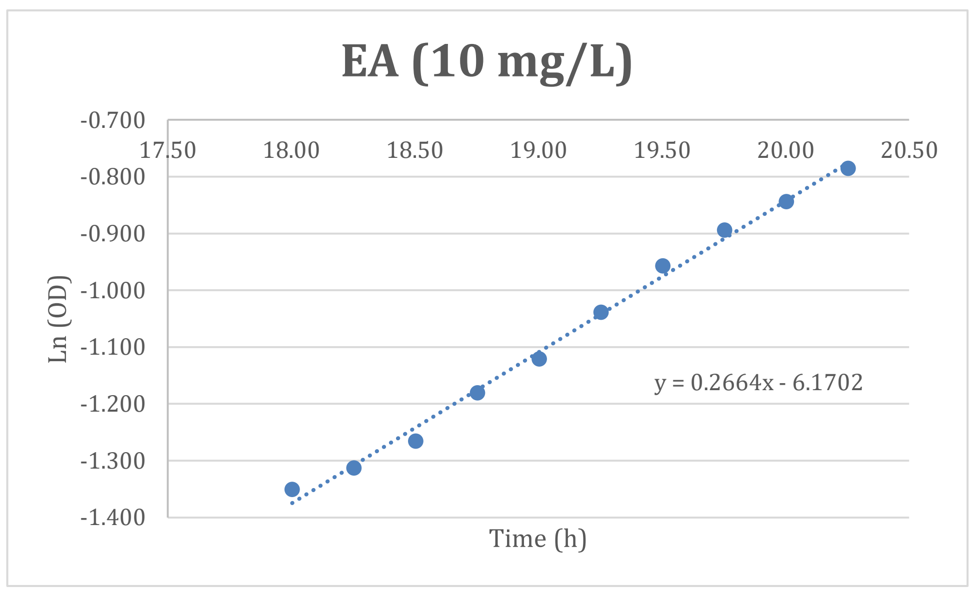

Figure 42: ***determination of μ for Ethyl Acetate (10 mg/L)***

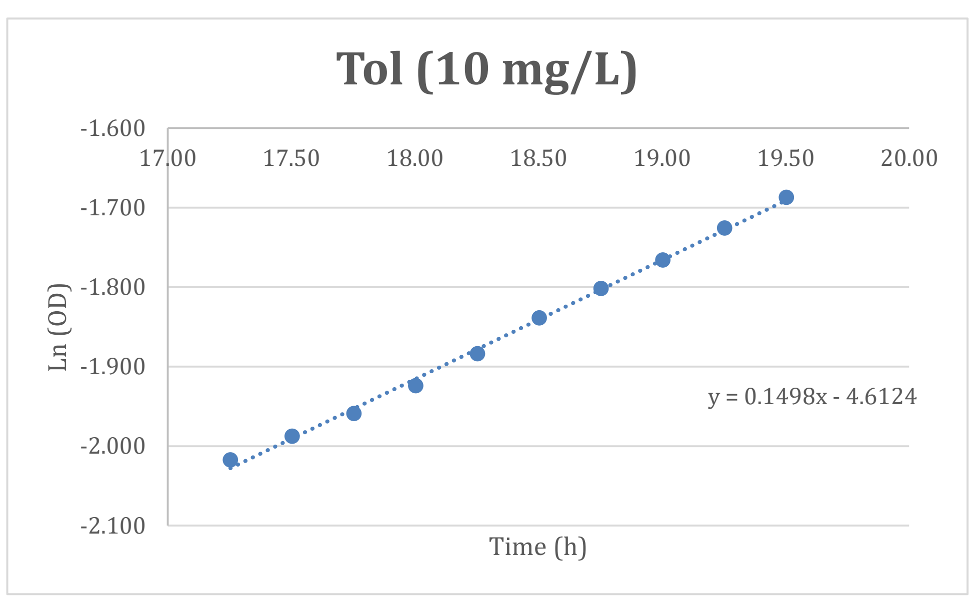

Figure 43: ***determination of μ for Toluene (10 mg/L)***

Annex 4. Laboratory-Scale Bioreactor

Figure 44: Lab scale bioreactor

Annex 5. Biofilm formation on the Carrier Material

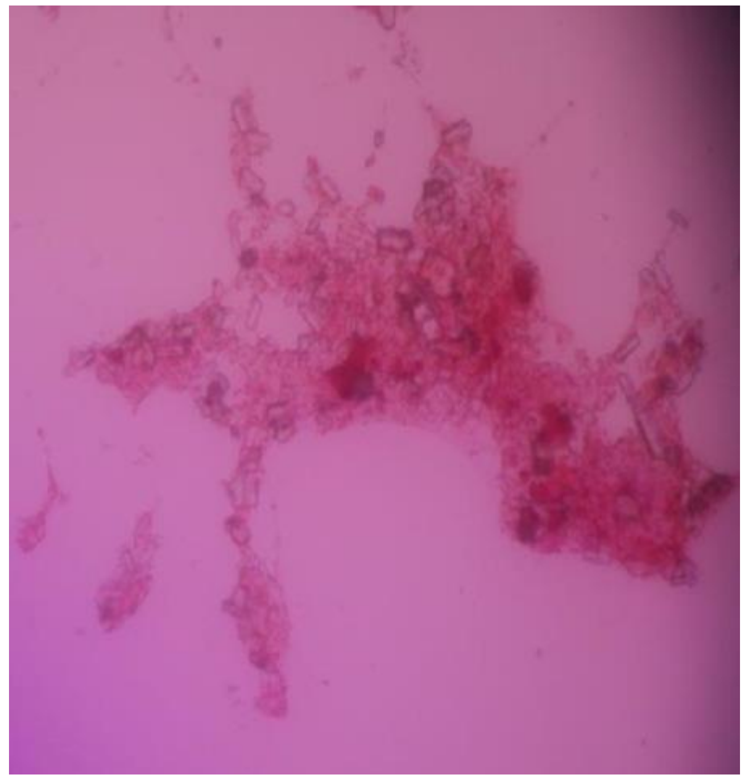

Figure 45: Microscopic analysis of immobilized microorganisms PUR A carrier material and after 5 days of operation.

Figure 46: Comparison between populated and pure carrier – PUR A foam after 5 days of operation.

Annex 6. Bioreactor Data

Table 11: Outlet concentration, Elimination Capacity and Removal Efficiency of the pollutants

Pollutant	0.66 $g.m^{-3}$			1.10 $g.m^{-3}$		
	C_{out} ($g.m^{-3}$)	EC ($g.m^{-3}.h^{-1}$)	RE (%)	C_{out} ($g.m^{-3}$)	EC ($g.m^{-3}.h^{-1}$)	RE (%)
Acetone	0.59	3.06	10.12	1.00	4.51	8.93
Dimethyl sulfide	0.53	5.80	19.14	0.89	9.44	18.70
Ethyl acetate	0.07	27.01	89.20	0.06	47.73	94.59
Propionic acid	0.04	28.31	93.50	0.03	49.02	97.14
α-Pinene	0.32	15.82	52.24	0.76	15.54	30.79
Triethylamine	0.59	3.31	10.95	0.96	6.25	12.38