Gaseous BTEX Biofiltration: Experimental and Numerical Study of Dynamics, Substrate Interaction and Multiple Steady States

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Gaseous BTEX Biofiltration: Experimental and Numerical Study of Dynamics, Substrate Interaction and Multiple Steady States

by

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Abstract

Air pollution has a global impact on the environment and human health. In recent decades growing consciousness of air pollutants has led to a substantial decline in hazardous emissions. Nevertheless, air quality problems persist. A group of pollutants of particular concern are benzene, toluene, ethylbenzene and xylene, commonly referred to as BTEX. BTEX are known for their adverse effects on human health such as the carcinogenicity of benzene among others. Continuous development, improvement and exploring of new innovative control technologies are of great importance and striven for by researchers and industry. Biological methods such as biofilters are considered to be a sustainable and environmentally friendly technology.

Hence, the present dissertation investigated the employment of a promising microorganism, *Nocardia* sp., to treat BTEX in a biofilter as well as the experimental and computational study of different steady states. At an empty-bed residence time (EBRT) of 1.5 min and an inlet concentration between 0.05 – 0.14 g m\(^{-3}\) single benzene, toluene, ethylbenzene and *m*-xylene were removed with an efficiency of 100%, 93%, 96% and 87% respectively. With increasing inlet concentration, the removal efficiency (RE) declined, however an increase of EBRT generally resulted in higher RE. A similar trend was observed when BTEX were treated as a mixture and highest RE were achieved at low concentrations. In addition, the determination of kinetic parameters of the microorganism were carried out and the threshold substrate concentration for benzene and *m*-xylene were estimated.

The exploration of a possible jump of steady states were numerically examined by considering only the biofilm. Therefore, two independent computer simulations were
developed, which includes diffusion limitation and substrate degradation following Haldane kinetics. Results clearly indicate a jump of steady states in a very small range of inlet concentration and a distortion of prevailing Haldane kinetics. A further development of one model was carried out and aforementioned determined kinetic parameters were applied. This model correctly described the jump of steady states in an actual biofilter at a concentration change of 0.272 g m$^{-3}$. Obtained results are supported by experimental validation.
Acknowledgement

I would like to thank my supervisor, Dr. Alex De Visscher for his guidance while working towards this dissertation. His profound knowledge and attitude always motivated me. I also would like to thank my co-supervisor Dr. Arindom Sen for his motivating attitude and advices. Furthermore, a thank you to my supervisory committee members Dr. Lisa Gieg and Dr. Hector Siegler for your great support.

I also want to thank everyone who supported me along my studies.
Dedication

This thesis is dedicated to my spouse Lisa, who always supported and never gave up on me even in harsh times. And to our daughter Josefine, who lively prevented me to be governed by my academic pursuit and academia itself and showed me, that this is not all what matters.
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List of Symbols, Abbreviations and Nomenclature

A  biofilm specific surface area (m² biofilm m⁻³ biofilter)

a  decay rate of biomass (h⁻¹)

c_A  substrate concentration (g m⁻³)

C_e  exit substrate concentration in the gas phase (g m⁻³)

C_i  inlet substrate concentration in the gas phase (g m⁻³)

D_A  diffusion coefficient of substrate in the biofilm (m² h⁻¹)

E  enzyme

EBRT  empty bed residence time

EC  elimination capacity [g m⁻³ h⁻¹]

EPEA  Environmental Protection and Enhancement Act

ES  concentration of enzyme-substrate complex in the liquid phase (g m⁻³)

ES  enzyme-substrate complex

ES₂  enzyme-substrate complex

Et  concentration of total enzyme in the liquid phase (g m⁻³)

f_N  fraction of nitrogen content in toluene degrading microorganisms in compost (g g⁻¹ biomass)

H  Henry’s constant (dimensionless gas-liquid partition coefficient)

ILR  inlet loading rate [g m⁻³ h⁻¹]
K_{\text{min}, \text{N}} \text{ rate constant for nitrogen mineralization (h}^{-1})

K_{\text{uptake, N}} \text{ rate constant for nitrogen uptake (h}^{-1})

K_I \text{ kinetic constant for substrate inhibition in the liquid phase (g m}^{-3})

K_m \text{ Michaelis-Menten constant for substrate biodegradation in the liquid phase (g m}^{-3})

K_N \text{ Michaelis-Menten constant for nitrogen (g m}^{-3} \text{ water)}

MLR \text{ mass loading rate [g m}^{-3} \text{ h}^{-1}]

N \text{ number of Collocation Points}

N_{\text{inorg}} \text{ inorganic nitrogen content in compost (g m}^{-3} \text{ water)}

N_{\text{org}} \text{ organic nitrogen content in compost (g kg}^{-1} \text{ compost}_{\text{dw}})

P \text{ product}

Q \text{ volumetric flow rate of waste gas (m}^{-3} \text{ h}^{-1})

r_1, r_2, r_3, r_4, r_5 \text{ rates of enzymatic reactions (g m}^{-3} \text{ h}^{-1})

RE \text{ removal efficiency [%]}

S \text{ substrate}

US EPA \text{ United States Environmental Protection Agency}

V \text{ biofilter bed volume (m}^3)

V_{\text{max}} \text{ maximum reaction rate (g m}^{-3} \text{ biofilm h}^{-1})

X \text{ biomass concentration (g}_{\text{dw}} \text{ biomass kg}^{-1} \text{ compost}_{\text{dw}})
Y \quad \text{yield [g g}^{-1}\text{]} \\
\delta \quad \text{biofilm thickness (m)} \\
\varepsilon \quad \text{porosity of the packed bed (dimensionless)} \\
\mu_{\text{max}} \quad \text{maximum specific growth rate of biomass (h}^{-1}\text{)} \\
\mu_{\text{net}} \quad \text{et specific growth rate of biomass (h}^{-1}\text{)} \\
\rho_{\text{bio}} \quad \text{density of biofilm (g}^{\text{dw}}\text{biomass m}^{-3}\text{biofilm)} \\
\rho_{\text{bulk}} \quad \text{[kg compost m}^{-3}\text{biofilter]} \\
v \quad \text{superficial gas velocity (m h}^{-1}\text{)}
The worst thing I can be is the same as everybody else. I hate that.

Arnold Schwarzenegger
1 Introduction

In this Chapter an introduction about air pollutions is provided. Furthermore, the motivation and the aim of this dissertation are stated. To outline and clarify the contribution of each author, a statement of contribution is included. In addition, some remarks on the main part are given.

1.1 Air pollution

Air pollution can be defined as “the presence in the outdoor atmosphere of one or more contaminants, such as dust, fumes, gas, mist, odor, smoke or vapor in quantities, of characteristics, and of duration, such as to be injurious to human, plant, or property, or which unreasonably interferes with the comfortable enjoyment of life and property” [1]. The World Health Organization (WHO) defines it as “contamination of the indoor or outdoor environment by any chemical, physical or biological agent that modifies the natural characteristics of the atmosphere” [2].

Air emissions have decreased over the last decades as a result of human awareness of the severe health and environmental impacts of air pollution. However, concentrations of some air pollutants are still too high after released into the environment and air quality problems and potential negative impacts on the environment persist. Since air pollutants are possibly transported over long distances in the atmosphere, the adverse effects on human health and the environment arise in areas other than where the pollutants originated. Hence, air pollution is not a matter of concern to any single country or industry but rather is a global concern requiring every effort at reduction of air pollution before release.
1.2 Motivation

The treatment of emitted waste gases is of great concern as they often contain harmful substances. Once released, these substances can cause severe health problems for humans and detrimentally impact the environment. With the world’s population growing, increasing industrialization, increasing technology and living standards, the release of waste gases is likely to increase and hence the necessity of treating and regulating emissions are crucial. One of the concerning gaseous emissions are known as volatile organic compounds (VOCs) and benzene, toluene, ethylbenzene and xylene, commonly referred to as BTEX, are a group of VOCs. These compounds are released in various industries including oil and gas, pulp and paper, petrochemical, etc. Long-term exposure to BTEX can lead to several harmful effects on the nervous, digestive, kidney and respiratory system. Moreover benzene is considered carcinogenic to humans. Such consequences clearly identify the need to sufficiently treat BTEX before released and therefore, researchers and industry are striving to find cost-effective, sustainable and environmentally friendly alternatives for air pollution control technologies.

Biological waste gas treatment technologies such as biofiltration have been used for several decades to diminish odors and VOCs in industrially released waste gas streams. The contaminated waste stream flows through a filterbed material and the pollutants of interest are biodegraded by the adhered microorganisms. The biodegradation process is a natural mechanism of microorganisms to utilize the carbon source, provided in terms of the pollutant, for microbial cell growth. No further addition of additives or energy is needed and no unwanted byproducts are produced. Hence, a biofilter is considered to be a sustainable and more cost effective technology compared to its physico-chemical
counterparts. Despite the successful industrial applications and efficiencies, biofiltration still encounters limitations and is not fully explored.

In recent years, an emphasis on experimental and numerical fundamental research and further development of the biofiltration process were given. Single and mixed microorganism have been investigated under various environmental conditions, and evaluated based on their ability to treat VOCs such as BTEX. Certainly, environmental conditions have a great impact on the removal efficiency (RE) but also the interaction between different microorganisms need to be considered. An inhibitory effect among microorganisms could potentially lead to a decline in RE and consequently, the treated gas still contains harmful compounds. However, a beneficial impact might increase the RE. Since a variety of indigenous bacteria are present in unsterilized filter bed material (i.e. compost), the interaction between microbes is an important factor. In addition, the diffusion behavior of the pollutant from the gas phase into the biofilm is also important for successful operation. The diffusion behavior can be impacted by the biomass growth. With increasing biomass, the biomass layer will grow and the diffusion limitation be more pronounced. In addition, the substrate (e.g. toluene) degradation rate also influences the RE.
1.3 Aim of study

The present dissertation, aims to develop a viable and efficient biofilter to treat waste gases containing low concentrations of BTEX, as well as to devise computer simulations to aid biofilter designers and operators. Due to the aforementioned interaction between microorganisms, a BTEX degrading bacterium was examined for its performance in the presence of other bacteria in order to verify whether this specific microbe would be able to achieve sufficiently high activity and for its possible application in the industry. In addition, information about its resiliency and competitiveness towards other microbes were obtained. Hence, each biofilter trial was inoculated with bacteria comprising a BTEX and non-BTEX degraders. Biofilter trials were carried out for single BTEX compounds and a mixture of BTEX and kinetic parameters were estimated as well.

In addition, the numeric investigation of substrate degradation rate and diffusion limitation were conducted by two independent computer models. These models indicated the occurrence of multiple steady states in a biofilm of a toluene degrading biofilter. In addition, it revealed a falsified kinetics (i.e., a kinetics that looks like Michaelis-Menten kinetics but is Haldane kinetics with diffusion limitation). To further investigate the multiplicity of steady states, a more sophisticated computer model, in terms of its complexity, was developed, considering a whole toluene biodegrading biofilter. To connect the sophisticated simulation to an implementable model, not only were literature values applied, but previously determined kinetic parameters were also applied. To support the predicted findings, a lab-scale experimental trial of multiple steady states was carried out.
1.4 Outline of dissertation

In Chapter 1 a brief introduction about air pollution is provided as well as the motivation and the aim of this study. A statement of contribution and remarks are stated as well.

An introduction to possible sources of air pollutants and six common gaseous and particulate air emissions are provided in Chapter 2. In addition, it addresses the hazardous effects of VOCs and specifically BTEX on humans and the environment. To describe the biofilter performance and be able to compare different studies the pertinent and established terminology is defined in Chapter 2. In addition, a comprehensive literature review with an emphasis on biofiltration is given, providing insight into the involved mechanisms of mass transfer and biological aspects.

Experimental assessment of one BTEX degrading bacteria, Nocardia sp., was performed and is described in Chapter 3. For each single compound of BTEX one biofilter was used and inoculated with Nocardia sp. and two non-BTEX degrading bacteria. The influence of operational parameters such as empty bed residence time (EBRT) and inlet load (IL) on the reactor performance were investigated. The determination of kinetic parameters, $K_m$, $K_I$ and $V_{max}$ were carried out and the decay rate for benzene and m-xylene were estimated.

The further evaluation of Nocardia sp. was conducted in Chapter 4. Here a mixture of BTEX was blended with an air stream and treated by means of biofiltration. The impact of varying EBRT and IL on the biofilter performance was examined. In addition, the kinetic parameter $V_{max}$, $K_I$ and $K_m$ were estimated. Acquired results were compared to those of Chapter 3 when possible.
Chapter 5 explains the development of two independent simulation models (steady state and non-steady state) to describe multiple steady-states. The multiplicity of steady-states in a single biofilm was computed for a biofilm under aerobic conditions, with diffusion-limitation and substrate degradation following Haldane kinetics.

The further evaluation of steady states in a biofilter was conducted in Chapter 6. Experimental validation of different steady states was conducted. In addition, the non-steady state model mentioned in Chapter 5 was further developed resulting in a more sophisticated (higher complexity) biofilter model, which enables the prediction of an operating biofilter. Subsequently the developed computer simulation was validated against aforementioned experimental results. Furthermore, to support the findings the computer model was also validated against experimental trials conducted in Chapter 3.

Chapter 7 provides a general conclusion and perspectives for further research.
1.5 Statement of contributions

Chapters 3 to 6 of this dissertation will be submitted to peer-reviewed journals and, hence the contribution of the first author, Michael Süß, will be clarified. Michael Süß conceptualized and contrived this research, set up all experimental trials and laboratory equipment, performed each experiment, analyzed and interpreted the majority of the results, wrote the majority of the developed computer simulations codes, and wrote and revised this dissertation. Dr. Alex De Visscher as the principle supervisor and corresponding author of all manuscripts provided incitations for this research, partially participated in interpreting and analyzing the results and revised parts of the developed computer simulation codes and manuscripts. All other parts of the dissertation were written by Michael Süß and comments were provided by the supervisor and corresponding supervisor.

1.6 Remarks

In Chapter 3 to 6 the subchapters: introduction, materials and methods are similar since experiments were carried out with the same equipment. Hence, it is not necessary to read each introduction, materials and methods parts in order to understand each of the studies. The treated BTEX blend in the biofiltration studies only comprises m-xylene out of the three possible xylenes, since it is based on an actual waste gas stream. Hence, m-xylene was the only xylene used in experiments described in Chapter 3 and 4.
2 Literature review

This Chapter gives an introduction and review of biological air pollution control methods such as biotrickling filter, bioscrubber and biofilter. A comprehensive explanation of biotrickling filters and bioscrubbers is beyond the scope of this thesis and therefore only a short overview is provided and emphasis is placed on biofiltration.

2.1 Definition and sources of air pollution

Air pollution can generally be distinguished between particulate matter and gaseous pollutants. The latter pollutant can be further classified in six rubrics, as is done by Environmental and Climate Change Canada [3]. These six rubrics as well as additional pollutants, will be further discussed in this Chapter.

2.1.1 Particulate matter

The United States Environmental Protection Agency (US EPA) defines particulate matter, also called particle pollution and abbreviated as PM, as a mixture of solid particles and liquid droplets in the air [4]. A similar definition is used by the European Environmental Agency, were particulate matter stands for a collective name for fine solid or liquid particles added to the atmosphere by processes at the earth’s surfaces [5]. In addition it includes dust, smoke, soot, pollen and soil particles. Environmental and Climate Change Canada defines particulate matter as airborne particles in solid or liquid form [6]. A further common subdivision based on particle size is PM$_{10}$ and PM$_{2.5}$, were PM$_{10}$ accounts for particle that are 10 micrometers and smaller and PM$_{2.5}$ are particles smaller than 2.5
micrometers in diameter. A variety of emission sources exist, which are directly emitting PM.

The association of PM to health risk has been studied in the past. Husan-Chia Yang et al. [7] studied the effect of different PMs on the cardiovascular disease (CVD) and showed that PM$_{2.5}$ was significantly positively correlated with the number of outpatient visits for CVD during high air pollution events. Brook [8] summarized different studies and concluded that, short- and long- term exposure to PM air pollution is linked to an increasing risk of cardiovascular morbidity and mortality. Even inhalation of PM$_{2.5}$ for a few minutes can trigger myocardial infarctions, heart failure, strokes, etc. Other studies are available in terms of PM effect on human health as well, finding a correlation between PM exposure and adverse health development [9–11].

### 2.1.2 Gaseous pollutants

#### 2.1.2.1 VOC and BTEX

Volatile organic compounds (VOCs) are often defined as having a vapor pressure of 0.01 kPa at 293.15K and comprising a variety of compounds such as alkanes, aromatic molecules, ketones, terpenes and sulphuric molecules [12]. Some definitions add that a VOC should participate in photochemical reactions in the atmosphere, which does not hold true for all VOCs. Various anthropogenic sources like industrial processes such as oil refining and petrochemical manufacturing as well as vegetable oil production, fish processing, flavor and fragrant manufacture, livestock air, hatcheries, laminate production, wastewater treatment plants, etc. might emit VOCs. In addition, biogenic released VOCs
contribute to the overall emissions, whereas plants emit a range of VOCs including alkenes, ketones and aldehydes, through their biochemical pathways.

In atmospheric chemistry VOCs have a crucial impact on the formation of secondary pollutant such as ozone and particulate organic matter. It also has an impact on the OH-radicals in the atmosphere, which potentially determine the residence time of other pollutants.

The exerted effects of VOCs on human health might be highly different dependent on the VOC. They potentially irritate the eyes, nose and throat, detrimentally impact the central nervous system, or are carcinogenic (e.g. benzene). Since VOCs are present in paints, varnishes, waxes, glues, cleaners, furniture and other products, they are found in indoor air as well. Methane per definition is a VOC but is often excluded from the list of VOCs and referred to as methane VOC. Hence, methane VOC is a specific pollutant contrary to non-methane VOCs (NMVOCs). Methane does not participate in photochemical reactions in the atmosphere, but it is considered to be a greenhouse gas (GHG).

The contribution of VOCs to the overall Canadian gaseous emissions are about 18% [12]. Benzene, toluene, ethylbenzene and m-xylene are aromatic hydrocarbons, referred as to BTEX. Anthropogenic sources for BTEX include processing of petroleum products and production of consumer goods such as pharmaceuticals, cosmetics, lacquers and rubber product. Despite the range of application and associated benefits and value for humans, these compounds have several health effects such as adverse impact on the central nervous system etc. The International Agency for Research on Cancer concluded, based on sufficient evidence, the carcinogenicity of benzene on human [13]. Such severe health impacts are reasons for the necessity of treating air streams contaminated with BTEX.
The Alberta Environmental Protection and Enhancement Act (EPEA) regulates Alberta’s ambient air quality objectives and guidelines [14]. For benzene, ethylbenzene, toluene and xylenes a one hour average maximum ambient concentration of 30 μg m⁻³, 2000 μg m⁻³, 1880 μg m⁻³ and 2300 μg m⁻³ are established [14]. The concentration at ground level need to meet regulations and usually an air dispersion model is used to conduct calculations. An important factor in regard to the harmful health impact of a compound and the severity of such a health impact are the exposure time and concentration. Based on these factors, the impact on human health can differ.

The Occupational Safety and Health Administration (OSHA) regulates the maximum benzene concentration in a workroom over an 8 hours workday at 1 ppm [15]. At a short exposure time of 5 – 10 min to a high benzene concentrations range of 10,000 – 20,000 ppm can be lethal. At levels between 700 – 3,000 ppm, benzene can cause drowsiness, dizziness, confusion, etc. [15]. In addition, the lifetime risk of leukemia at a benzene air concentration of 17 μg m⁻³ was estimated at 1 out of 10,000 [16]. An average of 200 ppm for toluene in air over an 8 hour workday was established by the OSHA, however the American Conference of Governmental Hygienists (ACGIH) recommends a threshold of 20 ppm [17]. At an acute concentration of 2,000 – 5,000 ppm ethylbenzene in air, dizziness has been observed [18]. OSHA sets the maximum allowable concentration of xylene in air to 100 ppm during an 8 hour workday [19]. Furthermore, at a xylene concentration at around 10,000 ppm for several hours exposure, one study reported an lethal incidence [19].

Several methods to treat VOCs and hence BTEX are commonly available and applied in industry. Combustion, plasma treatment, chemical precipitation and adsorption among others are physical-chemical methods. In contrast, biological methods e.g. biofilter,
biotrickling filter and bioscrubber among others based on the microbial capability to degrade the contaminant of interest are widely employed. The merits of biological treatment technologies are the reduced investment and running costs as well as the lack of undesired production of by-products.

2.1.2.2 Carbon monoxide and carbon dioxide

Carbon monoxide (CO) is an odorless and colorless gas, which can be released by motor vehicles or incinerators for example. Low level exposure may cause headache, fatigue and flu-like symptoms. However, exposure to higher levels of CO can cause severe health effects such as hypoxemia and tissue hypoxia, since it attaches to the oxygen transporter hemoglobin and therefore oxygen can not bind to it anymore.

CO₂ is a colorless gas and may cause suffocation, headache, visual and gearing dysfunction and unconsciousness. It is naturally present in the atmosphere as part of the carbon cycle, however anthropogenically released CO₂ alters the carbon cycle by adding more CO₂ and by impacting the natural sinks. Carbon dioxide can be released by burning of fossil fuels, solid waste, manufacturing of cement, etc. The natural sequestration of atmospheric CO₂ occurs by the adsorption by plants and microorganism as a part of their metabolic pathways or by the absorption by water bodies. Carbon dioxide is also considered to be a primary greenhouse gas (GHG) emitted through human activities [20], which is associated to the effect of global warming.
2.1.2.3 Sulphur oxides

Sulphur oxides may refer to diverse species of molecules comprising oxygen and sulfur such as sulphur monoxide (SO), sulphur dioxide (SO\textsubscript{2}), sulphur trioxide (SO\textsubscript{3}), disulphur monoxide (S\textsubscript{2}O), disulphur dioxide (S\textsubscript{2}O\textsubscript{2}) among others.

Sulphur dioxide (SO\textsubscript{2}) is a colorless gas with an irritating, unpleasant odour. SO\textsubscript{2} emissions originate from combustion processes of fossil fuels such as coal, vehicle exhaust gases and from volcanoes. Exposure to SO\textsubscript{2} has been associated with reduced lung function, adverse effects on the respiratory symptoms and irritation of the eyes, nose and throat. In the atmosphere, SO\textsubscript{2} reacts with other substances and potentially forms sulfate aerosols. Such aerosols, and especially fine particulate matter, can be carried into the pulmonary system and interfere with normal functionality. In addition, SO\textsubscript{2} emissions cause severe impact on the environment by influencing the habitat suitability for plant communities and animal life. If absorbed in water bodies it can unbalance the ecosystem by lowering the pH. Furthermore, it accelerates the corrosion of iron, steel and zinc by a reaction with moisture on the materials surface. Sulphur oxide is also a precursor of acid rain.

Sulphur trioxide vapor has no odour but is very corrosive and categorized as potentially carcinogenic. As a gas, SO\textsubscript{3} will be formed from SO\textsubscript{2} and further reacts with water to form sulfuric acid, which might dissolve in water and is removed from the air by rain.

2.1.2.4 Nitrogen oxides

Among the different air pollutants, forms of nitrogen and oxygen, nitric oxide, also referred to as nitrogen monoxide (NO) and nitrogen dioxide (NO\textsubscript{2}) are considered to be the major
pollutants in this rubric. Other pollutants are nitrate (NO$_3^-$), nitrous oxide (N$_2$O), dinitrogen trioxide (N$_2$O$_3$), dinitrogen tetroxide (N$_2$O$_4$) and dinitrogen pentoxide (N$_2$O$_5$). NO$_x$ is a commonly used symbol and refers to both NO and NO$_2$. NO$_x$ is emitted through combustion processes, but not limited to this source. If it reacts with moisture it can form small particles. In addition, it can react with hydrocarbons and oxygen under ultraviolet (UV) radiation and form photochemical smog. Adverse health effects such as eye and skin irritation and detrimental impact on the respiratory system are common. Emitted NO$_2$ can potentially react with hydroxyl radicals in the atmosphere and form nitric acid (HNO$_3$) and potentially lead to acid rain. In addition, nitrogen dioxide leads to unwanted ground-level ozone (O$_3$) as a temperature dependent reaction in the lower atmosphere. Yet a fraction of the formed ozone will react with NO to from NO$_2$, which eventually is again available to form ozone.

Nitrous oxide, also known as laughing gas, is a greenhouse gas (GHG) similar to carbon dioxide (CO$_2$) and methane (CH$_4$). CO$_2$ and CH$_4$ are known to have a major impact on global warming due to its global warming potential, which is the estimation of a pollutants ability to trap heat or infrared radiation reflected by the Earth’s surface. However, nitrous oxide is considered to have a 300 times higher global warming potential. A major cause of anthropogenically emitted N$_2$O are the nitrification of ammonium based fertilizer or denitrification of NO$_3$ in soils.

Another nitrogen containing pollutant is ammonia (NH$_3$). Agricultural activities such as livestock operation and hence the microbial degradation of manure is a major contribution to this emission. Furthermore, the use of nitrogen enriched fertilizers also play a significant
role, since not all of the nitrogen is consumed by the crops and therefore, the rest will be available for microbial degradation.

2.1.2.5 Odours

There are many different anthropogenic and biotic sources for odours such as refineries, industrial factories, livestock operation, agricultural sources, sewage and water treatment plants, lagoons, wildfires etc. An odorant or aroma compound is a chemical compound, which is sufficiently volatile and dissolved in the ambient air to be transported to the olfactory system of a human. There the cognition of fragrance, pleasant or unpleasant, will occur. Odorants encompass a wide range of compounds, e.g. alcohols, aromatics, sulphur compounds, acids, etc. and greatly vary in size, structure and functional groups.

2.1.2.6 Ozone

Ozone (O₃) is a colorless gas and is formed as a result of a series of chemical reaction between VOCs, nitrogen oxides and oxygen under solar ultraviolet (UV) irradiation. The exposure to ground level (troposphere), can cause adverse health effects such as asthma or other respiratory harms, while ozone in the stratosphere is desirable since it is capable of filtering UV radiation.
2.2 Biological air pollution control

Various technologies are available to treat air pollution and can be distinguished by physical-chemical methods such as combustion, plasma treatment, chemical precipitation, absorption, etc.; and biological technologies. Most commonly biological systems are used to treat odour and VOCs. The biological treatment methods that are most commonly used are biofiltration (BG), biotrickling filtration (BTF) and bioscrubbing (BS), as schematically shown in Figure 2.1. Crucial for biological methods is that microorganisms are biodegrading the pollutant of interest. The contaminated gas stream flows through a medium containing microorganisms and due to the pollutant transfer from the gas phase into the liquid phase the molecules are available for biodegradation. As a result mostly heat, H₂O and CO₂ are produced. However, other products such as acids or alcohols can be produced as well and in some applications are desired.

Biotrickling filters are very similar to biofilters; both consist of a reaction vessel filled with a fixed filter bed and adhered microorganisms. The main difference is that the filter bed of a BTF is continuously irrigated with an aqueous solution that may contain nutrients. Due to the permanent supply of liquid, BTF are more adapted to treat more water soluble VOCs. Typical inlet concentrations for a biotrickling filter are below 0.5 g m⁻³ [21], although higher concentrations were treated as well [22–25]. Process parameters such as pH and moisture content are easier to control, due to the continuous feeding of liquid. The drawback to the system is the excessive accumulation of biomass and therefore a significantly increased pressure drop can occur [26].

A bioscrubber consists of an absorption and bioreactor unit. In the absorption unit, which could contain a packing material the gas and liquid phase flow in counter-current direction.
Consequently, the gas pollutant is transferred into the liquid phase and is pumped and agitated into the bioreactor unit. The bioreactor unit contains microorganisms suspended in the aqueous phase biodegrading the pollutant molecules. The main advantage of a bioscrubber is a stable operation and better control of operating parameters as compared to other biological methods. Treatment of soluble VOCs with a Henry constant < 0.01 is possible.

Figure 2.1: Schematic of a biofilter in a), biotrickling filter in b) and a bioscrubber in c); 1) polluted air, 2) biofilter, biotrickling filter or adsorption unit, 3) treated air, 4) nutrient solution, 5) irrigation, 6) bioreactor unit and 7) discharge
2.3 Biofiltration

Biofilters were initially designed to treat odorous compounds from wastewater treatment plants and other anthropogenic sources. Since the first application the interest in this method has grown. The reactors are designed as an open, porous filter bed equipped with a proper air distribution system and sometimes including an irrigation system. The contaminated gas stream flows through a packing material adhered with microorganisms biodegrading the compound of interest. Biofiltration is considered to be a low-cost technology to treat gaseous VOCs and odor nuisance compared to other technologies such as incineration, absorption, non-thermal plasma, etc. and has been implemented in industrial processes [27].

Performance parameters are used to assess a biofilter and are referred to in biofiltration terminology. A series of complex mechanisms occur during biodegradation of gas-phase pollutants. In general biofiltration is a two step process. In the first step the pollutant is transferred from the gas phase to the surface of the biofilm, which is considered to be liquid. Secondly, bio-oxidation of the adsorbed pollutant occurs by the microorganisms present in the filter bed material/biofilm.

2.4 Terminology of biofiltration

In order to clearly understand biofilter operation and estimate biofiltration performance, general terminology pertinent to the field is defined. In the following the empty bed residence time (EBRT), volumetric loading rate (VLR), mass loading rate (MLR), removal efficiency (RE), elimination capacity (EC) and CO₂ production rate (P_CO₂) are clarified.
2.4.1 Empty bed residence time

The EBRT relates the gas flow rate to the volume occupied by the filter bed material [26,28,29].

\[ EBRT = \frac{V}{Q} \] (2.1)

Where V is the volume of the filter bed material [m\(^3\)] and Q is the gas flow rate [m\(^3\) h\(^{-1}\)]. Since the volume of the filter bed material used for calculating the EBRT is larger than the actual volume of the gas phase available, the computed results are overestimating the actual residence time. In order to calculate the theoretical actual residence time of the pollutant (\(\tau\)) the void space of the filter bed material (\(\theta\)) is needed [26,28]. Therefore,

\[ \theta = \frac{V_v}{V_T} \] (2.2)

\[ \tau = \frac{V_T \theta}{Q} \] (2.3)

where \(V_v\), \(V_T\) and \(\theta\) represent the volume of the void space [m\(^3\)], the volume of the filter bed [m\(^3\)] and the porosity of the filter bed material [26,28,29]. The accurate determination of the filter bed porosity is often arduous and it will change because of the biofilm built up over time. Hence, the simpler definition, equation (2.1), is commonly used.

2.4.2 Volumetric loading rate

The volumetric loading rate (VLR) is the ratio of gas flow rate [m\(^3\) h\(^{-1}\)] to the volume of the filter bed [m\(^3\)] and is defined as follows [26,28,29]:

\[ VLR = \frac{Q}{V_T} \] (2.4)
2.4.3 Mass loading rate or inlet loading rate

The mass of pollutant [g m\(^{-3}\)] entering the biofilter per unit time [h] and unit volume [m\(^3\)] of filter bed material is defined as mass loading rate (MLR) or inlet loading rate (ILR) and can be expressed as follows [26,28,29]:

\[
\text{MLR} = \frac{Q C_i}{V_T} \tag{2.5}
\]

where \(C_i\) represents the inlet pollutant concentration [g m\(^{-3}\)]. This performance parameter declines along the biofilter height as the pollutant is gradually bio-degraded, based on the ability of the microbes to utilize the contaminant.

2.4.4 Removal efficiency

Removal efficiency (RE) is the fraction of the biodegraded pollutant expressed as a percentage. It is defined as follows:

\[
\text{RE} = \frac{(C_i - C_o)}{C_i} \times 100 \tag{2.6}
\]

where \(C_o\) is the outlet concentration of the pollutant [g m\(^{-3}\)]. The RE reflects the specific conditions under which it is measured and differs with varying inlet concentrations of pollutant, gas flow rate and biofilter size [26,28,29].

2.4.5 Elimination capacity

Elimination capacity (EC) is defined as the mass of pollutant degraded per unit volume of filter bed material per unit time. This parameter is defined as follows:
EC = \frac{Q (C_t-C_o)}{V_T} \quad (2.7)

EC can reach a maximum equal to the MLR. At low inlet concentration, the EC will likely equal the MLR and the system will reach a 100% RE. With increasing MLR a threshold will be reached where the EC will be smaller than the MLR, which corresponds to RE < 100% and is called the critical elimination capacity or critical load [26,28,29].

### 2.4.6 CO₂ production rate

Complete mineralization of pollutants and biodegradation within the biofilter is commonly assessed by measuring and calculating the carbon dioxide production rate (P\textsubscript{CO₂}). Since CO₂ might be added within the biofilter system, removed through endogenous respiration, or may also be used as a carbon source by autotrophic bacteria, the mass balance calculation will not always be accurate. However, the CO₂ production rate can be calculated as follows [26,29]:

\[ P_{\text{CO}_2} = \frac{Q (\text{CO}_2\text{out}-\text{CO}_2\text{in})}{V_T} \quad (2.8) \]

### 2.5 Mechanism of operation

Biofiltration of VOCs is a complex series of physicochemical and biological mechanisms. Although the mechanisms of VOC biofiltration is mostly unknown, it is assumed that it starts with diffusion of the pollutant vapor and oxygen from the waste gas towards the biofilm which subsequently diffuses into the biofilm where it is biodegraded. The
following sections elucidate concepts related to the mechanism of VOCs to describe the biofiltration process.

2.5.1 Transfer and partition of pollutant

The first step, the transfer of contaminants from the gas phase to the liquid phase, is generally not a rate-limiting step and is related to Henry’s law constant \( (H_c) \). Henry’s law states that pollutant concentration in the gas phase is proportional to its concentration in the liquid phase [30] and is described as follows:

\[
H_c = \frac{C_g}{C_l} \quad (2.9)
\]

where \( C_g \) and \( C_l \) are the VOC concentration in the gas phase and liquid phase \([\text{g m}^{-3}]\) respectively. However, Henry’s law constant can be described in different units widely used in literature [31–34]. When dimensionless Henry’s law constant is used, the solubility of a substance described with \( H_c \) increases as \( H_c \) gets smaller, since an increasing number of Henry’s law constant indicates a partition towards the gas phase.

2.5.2 Diffusion of pollutant

The diffusion mechanism of a pollutant through a biofilm is well established, considering the good fit between developed computer simulation models and experimental verification. However, diffusion depends on different factors e.g. pollutant and temperature among others. The thickness of a biofilm is an important factor affecting the efficiency of biodegradation. A thick biofilm may pose mass transfer limitation, however a higher
microbial population occurs and consequently leads to a higher conversion rate. On the other hand, a thin biofilm is not a limiting factor in terms of mass transfer, but a lower microbial population in the biofilm leads to lower removal rates.

Due to microbial proliferation, the biofilm thickness is not constant and grows over time. Considering the need of substrate for biomass growth and eventually an increase of biofilm thickness, the substrate degradation has a faster dynamics than the biofilm growth. In other words, the time scale of the biodegradation of substrate molecules in a biofilter is on the order of a minute, which is orders of magnitude shorter than the biofilm growth which has a time scale of days. Therefore, the system is commonly assumed to be in a quasi-steady state, in terms of its biofilm growth for short periods of time. Hence, Fick’s second law of diffusion is used to describe the pollutant diffusion through a biofilm and is described as follows:

\[
\frac{dc}{dx^2} = \frac{r}{D}
\]

(2.10)

where the thickness of the biofilm is denoted with \( dx \) [m], \( D \) represents the diffusion coefficient of pollutant through the biofilm [\( m^2 \text{ s}^{-1} \)], \( s \) denotes the substrate concentration in the liquid [\( g \text{ m}^{-3} \)] and \( r \) expresses the substrate degradation rate [\( g \text{ m}^{-3} \text{ s}^{-1} \)].

2.6 Governing factors affecting biofiltration performance

Factors affecting the biofiltration performance are discussed in this section and distinguishes the biological factors from others. Among all these factors the inlet concentration load and the waste gas composition were changed in the conducted experiments (Chapter 3, 4 and 6), ranging from 0.050 g m\(^{-3}\) to 1.5 g m\(^{-3}\). In addition, the
flow rate through the biofilter was adjusted (1.5 min, 2.5 min, 4.5 min) in order to test the effect of EBRT on the system. The temperature was kept constant, the packing material was not changed and factors like pH, pressure drop and water content were monitored.

2.6.1 Biological factors affecting biofiltration

Biological factors are crucial to maintaining biofilter operation. A comprehensive elucidation and review of basic biological mechanism are beyond the scope of this thesis. However, an overview of necessary nutrients, bioavailability and microbial population will be discussed.

2.6.1.1 Nutrient availability

The pollutants represent the major carbon and energy sources for microbial activity. Hydrogen and oxygen are generally available in the air and sometimes in the filter bed material or in the VOC composition. The availability of other macronutrients such as nitrogen (N), phosphorus (P), potassium (K), sulphur (S) and others and micronutrients like vitamins and metals, are often present when an organic filter medium is used in the biofilter. When an inorganic material is used as filter bed material necessary nutrients can be added by feeding an aqueous phase. It is desirable to provide sufficient enough nutrients, since nutrient consumption and biomass growth can potentially change the spatial distribution of proliferating microorganism over time. A progressive nutrient deficiency can turn into a limiting factor for the long-term biofiltration performance [35].
With biomass growth the depletion of nitrogen becomes more pronounced and depletion zones shift steadily further into the biofilter [36]. Conversely, microorganisms also produce organic nitrogen due to cellular growth, and during cell death organic nitrogen is converted to ammonia. This ammonia is volatilized, nitrified and assimilated into new cells or can be denitrified to nitrogen gas [36]. The depletion of nitrogen in the biofilter can be described by two mechanisms proposed by Song [37] and are as follows: uptake of nitrogen for microbial growth \( (N_{\text{uptake}}) \) and leaching of nitrogen from the biofilter \( (N_{\text{leachate}}) \)

\[
\Delta N_{\text{media}} = N_{\text{recycled}} - N_{\text{uptake}} - N_{\text{leachate}}
\]

where \( \Delta N_{\text{media}} \), \( N_{\text{recycled}} \), \( N_{\text{uptake}} \) and \( N_{\text{leachate}} \) are the change in the inorganic nitrogen \( (\text{NH}_4^+ + \text{NO}_3^-) \) content \( [\text{mg}_N \text{ day}^{-1}] \), the inorganic equivalent of organic nitrogen recycled \( [\text{mg}_N \text{ day}^{-1}] \), the assimilated inorganic nitrogen of the biomass \( [\text{mg}_N \text{ day}^{-1}] \) and the biofilter leachate containing inorganic nitrogen, respectively \( [\text{mg}_N \text{ day}^{-1}] \).

The use of different carbon sources, glycerol, 1-hexanol, wheat bran and \( n \)-hexane, to enhance the startup time of a fungal biofilter treating \( n \)-hexane was investigated [38]. Cylindrical glass columns (1m) with an inner diameter of 0.07 m filled with dry perlite equal to a volume of 2.4 L were used at 30°C to conduct experiments. An EBRT of 1.3 min and an ILR of 325 g m\(^{-3}\) h\(^{-1}\) were used. The intent was to use one of the mentioned alternative carbon sources before hexane polluted air was introduced, except for the control biofilter were hexane was introduced from day 0. Results showed that the adaptation period decreased from 36 days to 7 days when wheat bran was used and reached maximum EC of 160 g m\(^{-3}\) h\(^{-1}\). For glycerol and 1-hexanol the adaptation period reduced to 24 days and 14 days, respectively.
2.6.1.2 Bioavailability

Among other factors to consider in order to ensure the effective biodegradation of pollutants in gas streams, it is critical to establish the bioavailability of such molecules. Bioavailability is a function of pollutant uptake by the microbial population and mass transfer mechanism and hence influenced by various factors, such as desorption, diffusion and dissolution. Due to long term contamination of soil, a decrease in bioavailability can occur and chemical or biological surfactants can improve the bioavailability. Surfactants can be subdivided into two categories: chemical and biological surfactants. Both lower the surface and interfacial tensions at a phase interface. Surfactants can be further subdivided into anionic, cationic, non-ionic and dual charge [39]. The ability to emulsify two compounds due to surfactants in aqueous solutions increase the bioavailability of hydrophobic or insoluble organic compounds [40,41]. The advantages of using biosurfactants instead of chemical agents for biodegradation of VOCs are the lower toxicity and environmental benefit of using natural products [42]. Based on these advantages biosurfactants have been studied to improve solubility and bioavailability of hydrocarbons in soils [43,44], and in gas biofiltration as well [45–47].

2.6.2 Concentration load

The concentration load entering the biofilter has a major effect on the operation of the system. Biofilters may treat MLR ranging from < 0.1 g m\(^{-3}\) h\(^{-1}\) to 100 g m\(^{-3}\) h\(^{-1}\) [28] and hence results of experiments and operations need to be properly interpreted in terms of the load being treated in addition to other factors. Inlet concentrations between 0.5 - 5 g m\(^{-3}\) have been reported as optimum for VOC biofiltration [48–51]. Biofilters vary in size
depending on inlet concentration and ML, in order to achieve required EC and RE. A pollutant can be introduced into a biofilter by either high concentration in a low volumetric loading rate or low concentration in a high volumetric loading rate [28]. With respect to pollutant diffusion through the biofilm, a possibility for overcoming diffusion limitation might be to provide a higher concentration at low surface loading rates, which in turn would cause a high pollutant concentration in the biofilm and increased biodegradation. This holds true if the biodegradation rate following reaction order higher than zero [28].

Lab-scale experiments are often carried out by varying the inlet concentration from low to high values at constant EBRT or by maintaining a constant inlet concentration and varying the EBRT [29,52,53]. In addition, the effect of transient inlet loadings and shock loads on the biofiltration system are often verified [54–57].

A compost/ceramic biofilter inoculated with a microbial consortium obtained from a sewage treatment plant was investigated for its capability to treat toluene and xylene contaminated air [58]. The bioreactor made out of poly-acrylic tubes with a diameter of 5 cm and a height of 70 cm was packed with filter material to a height of 50 cm. The ILR varied between 7.5 g m$^{-3}$ h$^{-1}$ and 213.2 g m$^{-3}$ h$^{-1}$. Maximum EC for toluene and xylene were 55.2 g m$^{-3}$ h$^{-1}$ and 27.6 g m$^{-3}$ h$^{-1}$ at ILR of 111.1 g m$^{-3}$ h$^{-1}$ and 95.2 g m$^{-3}$ h$^{-1}$, respectively. The authors concluded that the biofilter could handle a fluctuating operation based on the 30 days starvation and recovery of the system. This indicates a potential application on bigger scales. Amin et al. studied the biodegradation of $n$-hexane as a single pollutant and in mixture with BTEX [59]. The scoria/compost biofilter was made of a stainless steel column with an inner diameter of 11.5 cm, a height of 140 cm and the volume of the filter bed material was 8.3 L. Single $n$-hexane EC$_{\text{max}}$ of 10.9 g m$^{-3}$ h$^{-1}$ was obtained for ILR of
14 g m⁻³ h⁻¹ at an EBRT of 138 s, which corresponds to an RE of 81.7 ± 1.4% for an inlet concentration of 0.36 ± 0.09 g m⁻³. With a decrease of EBRT to 108 s the ECₘₐₓ dropped to 8.1 g m⁻³ h⁻¹ for an ILR of 11.6 g m⁻³ h⁻¹, corresponding to an RE of 70.1% at an inlet concentration of 0.28 ± 0.05 g m⁻³. The introduction of BTEX compounds caused a significant decline of ECₘₐₓ down to 2.8 g m⁻³ h⁻¹ at ILR of 12.3 g m⁻³ h⁻¹ of n-hexane at constant EBRT of 108 s, which correspond to an RE of 21.4%. RE of benzene, toluene, ethylbenzene, m/p-xylenes and o-xylene were 90%, 98%, 98% and 90%, respectively. With a further decrease of EBRT to 83 s a more pronounced effect was observed for n-hexane, only reaching 15.1%. Individual BTEX removal rates were 85%, 94%, 97%, 89% and 66% for benzene, toluene, ethylbenzene, m/p-xylenes and o-xylene, respectively.

2.6.3 Waste gas composition

In lab-scale application, biofilters are often evaluated by a single pollutant entering the system [60–62], but mixtures of VOCs are also investigated [63,64]. After all, in industry the presence of multicomponent waste gases is more prevalent than a single component [65]. The competitive effect of the presence of multiples pollutants may interfere with mass transfer and/or biodegradation. In addition, more pronounced inhibition can occur if there is a preferential uptake of one pollutant over another, or if there are toxic interactions between the pollutants and microbes [42].

A characterization of the removal performance based on the compounds class was suggested by [66] and followed the sequence alcohols > esters > ketones > aromatics > alkanes. Balasubramanian et al. [67] reported similar results where alcohols biodegraded within 30 cm of a 100 cm high biotrickling filter and an aromatic compound (toluene) was
biodegraded in higher portions of the column. Toluene, along with chloroform were the only compounds still detectable under ILR of chloroform of up to 14.22 g m\(^{-3}\) h\(^{-1}\). With increasing chloroform concentration, the RE of VOC did not have significant impact, except for toluene and chloroform with 16% and 23% in the outlet stream.

### 2.6.4 Packing media

The packing media, also referred to as filter bed material, can be defined as a solid medium on which microorganisms adhere and eventually form a biofilm. It is the main part of the biofilter and biodegradation of pollutants occurs here. Therefore, it is crucial to select the most suitable filter bed material to optimize the biofilter performance and stability. Different physicochemical properties of the packing material need to be considered; for instance, 1) high specific surface area to facilitate gas-biofilm mass transfer, 2) high porosity for homogeneous distribution of the gas, clogging prevention and microbial growth, 3) good water retention capacity to avoid bed drying, 4) availability of nutrients and 5) structural integrity and ability to resist filter bed compacting due to operation [26,39,68].

The packing media can be an organic natural or inorganic synthetic material [26]. Examples of organic materials are compost, peat, soil and wood bark/chips. Organic packing materials are used most often, since they possess a lot of the desirable criteria and are generally available for low costs. However, each material has it merits and drawbacks. The availability of essential nutrients, a variety of indigenous microbes, a good water holding capacity and good air permeability are advantages to using compost [50], however it is often less stable than soil and peat with a tendency to break down and consequently
increase the pressure drop over time. The EC of compost biofilters is inversely proportional to the particle size, implying that for biofilter activity the active surface area of support particles is an important factor [69]. In addition, the composition of compost is likely to vary depending on the nature of the substrates. Uneven water distribution can occur as a result of deterioration of the organic packing material, which decreases the bioavailability of VOC and reduces the lifespan of the filter bed, even if the organic matter is abundant and affordable [42]. Inorganic material such as ceramics, polyurethane foam, glass wool, etc, are usually more stable since no biological deterioration occurs and the gas distribution is more uniform due to its defined structure. However, the addition of nutrients may be required and the materials are generally more expensive [70]. One way to overcome the previously mentioned drawbacks might be the combination of both organic and inorganic material.

Sun et al. investigated the performance of a biotrickling filter with mixed organic and inert packing materials [71]. Inert ceramic raschig rings, ceramic pall rings and lava rock were used as structured mixed packing (SMP) material and mixed with a blend of coral rock, bark, ceramisite, charcoal and compost. Results showed highest EC for ceramic pall rings, followed by rashig rings and lava rocks, which implies that the best suited SMP materials are pall rings. A possible measure to reduce the probability of the filter bed crushing or compacting is the addition of materials such as wood chips, perlite, glass beads, etc. These materials delay clogging and increase filter bed lifespan. One biofilter packed with organic material, in this case composted leaves, and three filters with non-organic sponge-based material, blast furnace slag and expanded vermiculite were compared in terms of their CH$_4$ oxidation at low concentrations [72]. The biofilter packed with expanded vermiculite
showed the highest conversion rates at > 90%. Gaudin et al. [73] developed a packing material composed of calcium carbonate, an organic binder and two different nitrogen sources, ammonium phosphate and urea phosphate (UP). Each mixture of calcium carbonate, organic binder and one nitrogen source generated a basic pH, which may buffer the filter bed in case of acidic pH generation during biofiltration. Evaluation was based on bulk density, moisture retention capacities and water cohesion capacities among others. UP20 (UP with 20% organic binder) was chosen to be tested in a hydrogen sulfide biofilter. Results suggested that the new packing material has a significant advantage over pine bark or pozzolan at inlet concentration > 100 mg m$^{-3}$.

2.6.5 Temperature

Temperature is a factor that significantly affects the microbial activity and consequently the biofilter performance. A general distinction in microbial classes can be made based on ambient temperature as follows – psychrophilic microorganisms < 20°C, mesophilic microorganisms 20-40°C and thermophilic microorganisms > 40°C. An important consideration is that for an increase of about 10°C of temperature the biological activity roughly doubles until an optimum temperature range is reached [28,40]. The majority of studies are conducted with microorganisms which have their growth optimum in the mesophilic range, and the favorable temperature range for BTEX treatment is found to be between 20°C - 30°C [74–76]. For industrial application the waste gas stream needs to be cooled down, since depending on the nature of the process and product emitted waste gas streams might exceed temperatures of 40-50°C. Pre-cooling of such emissions to a mesophilic temperature range can increase the investment and operational cost. The
potential increase of cost and the rise of biological activity would favor the use of thermophilic organisms. In recent years thermophilic biofilters were studied.

Hu et al. [77] carried out biofiltration of toluene, benzene and hexane in a stainless steel bioreactor with an internal diameter of 8 cm, porous perlite as filter bed material and a gas stream temperature of 50°C. Highest removal efficiencies of 95% and 90% were obtained for Toluene at lower (0.350 – 0.550 g m⁻³) and higher (0.700 – 1.200 g m⁻³) inlet concentrations under an EBRT of 27 s. Removal efficiencies for benzene and hexane, were 85% and 70% at lower inlet concentrations (0.350 – 0.550 g m⁻³ and 0.150 - 0.400 g m⁻³) and 70% and 50% at higher concentrations (0.800 – 1200 g m⁻³ and 0.550 – 0.950 g m⁻³), respectively. Moussavi et al. [78] evaluated a thermophilic biofilter in terms of its effectiveness and considered the system as effective treating MTBE in low and high concentrations without significant biomass accumulation. Balsam et al. [75] compared a mesophilic and thermophilic biofilter to treat BTEX contaminated air. They operated the mesophilic unit at around 20°C and the thermophilic unit at around 50°C. For both, the filter bed consisted of 4 L perlite with a diameter of 4 – 6 mm and an EBRT of 96 s. Based on the plot of elimination capacity of the mixture as a function of inlet load, the researchers obtained a fairly linear relationship between EC and BTEX loading rate for both units under inlet loads of about 150 g m⁻³ h⁻¹. They found the data to be more scattered at higher inlet loads. Highest EC of BTEX were 188 g m⁻³ h⁻¹ at loadings of 304 g m⁻³ h⁻¹ and 218 g m⁻³ h⁻¹ at loadings of 255 g m⁻³ h⁻¹ for the mesophilic and thermophilic biofilters, respectively. The authors indicated the possibility of using a thermophilic biofilter for such an application. In addition, the thermophilic unit showed higher EC and lower pressure drop compared to the mesophilic unit and a higher removal of benzene. However, it is
worth mentioning that studies showed a decrease in RE with increasing temperature, likely due to the lack of microbial adaptation and the reduced tolerance to substrate toxicity at elevated temperatures.

The thermophilic biofiltration of benzene and toluene and comparison to a similar mesophilic biofilter were conducted using a laboratory-scale biofilter made of square acrylic resin column (0.15 m x 0.15 m x 0.83 m) [79]. The experiment employed polyurethane as a packing material at a total volume of 6.75 L, and the operational temperature was around 60°C. Different space velocities (or superficial gas velocities) and inlet concentrations were tested. By comparing EC under consideration of EBRT the rate of benzene and toluene removal was similar at mesophilic and thermophilic temperatures. In this study other reports were found with analogous results, and therefore the authors concluded that there is no negative effect on the biodegradation of pollutants at elevated temperatures.

2.6.6 pH

The performance of a biofilter is affected by pH in several ways analogous to the effect of temperature. Based on the fact that microbial species activity varies under different pH, the highest performance is reached at different pH levels. Fluctuation in pH can lead to a shift in microbial proliferation, which does not necessarily mean the biodegradation rate will drop [26]. The bio-oxidation of certain compounds such as hydrogen sulfide, organic sulfur compounds and halogenated compounds, among others, potentially form acidic end products and lower the pH, consequently altering enzyme activity. The buffering capacity
of the filter bed material can prevent acid accumulation, which is the case with peat for instance.

When a nutrient solution was added at the top of a biofilter treating hydrogen sulfide, dimethyl disulfide and ethanethiol and packed with one layer of composted wood and one layer of pine bark, the pH remained relatively constant at the top (composted wood). However, a decrease from about 8.5 to 6.5 versus 7 to less than 3 was observed in lower layers without adding the nutrient solution [80]. Toluene biofiltration and pH behavior was evaluated in a 0.75 m high column with a diameter of 0.145 m divided into three equal stages and filled with a mix of compost and sea shells, in a ratio of 1:6 w/w [81]. In the period of 181 days the pH remained between 6.5 and 8, albeit with a decrease at the beginning (the first 20 – 30 days). Liangcheng et al. [82] investigated the effects of pH on gaseous ammonia removal in 4 plastic cylindrical biofilters with a height of 50 cm and an inner diameter of 45 cm. ILR remained at 5.46 g m$^{-3}$ h$^{-1}$ at EBRT of 16s. At an initial pH of 8 the RE was between 85% - 95% for 50 days. After adjusting the pH to 4.54 and 6.04, RE stabilized between 80% - 85%, while at a pH of 8.04 and 9.57, RE were 70% and 60%, respectively. The higher RE in acidified biofilters was possible due to the low moisture content during experiments. Two biofilters packed with reticulated polyurethane foam and inoculated with an undefined mixture of microorganisms treated a gas stream containing a blend of acetone, methyl ethyl ketone, toluene, ethylbenzene and p-xylene with an added acidic nutrient solution (pH 3) [83]. Results indicated that this microbial population was able to degrade a mixture of VOCs under acidic conditions, with RE ranging from 97% to 99%. However, the fungal-dominated biofilter showed a longer startup period compared to a neutral pH biofilter.
2.6.7 Moisture content

The moisture content of the filter bed material is an important factor for bio-oxidation of pollutants, since water is required by the thriving microbial population to preserve their metabolic activity. The water content can be maintained by adding water directly through a sprinkler from the top of the biofilter or by pre-humidifying the incoming gas. The latter option implies the importance of the relative humidity of the pre-humidified gas. By sprinkling water from the top it could be beneficial to introduce the gas stream co-currently since high bio-oxidation and consequently heat are produced near the gas inlet. Thus, drying of the top portion of the filter bed might be avoided.

It is critical to avert water losses that result from the temperature increase due to microbial activity and volatilization. Issues associated with low filter bed moisture can include 1) filter bed drying and 2) the development of fissures causing channeling and short circuiting, which lead to an alteration in the EBRT. On the other hand, a higher-than-optimal water content can potentially lead to 1) an increase of mass transfer limitation of oxygen and pollutant to the biofilm and causing the emergence of anaerobic zones and limiting the overall reaction rate, 2) change in EBRT, 3) increasing pressure drop due to the lack of void volume, 4) channeling of waste gas within the filter bed, 5) potential foul odor due to the lack of oxygen and 6) leaching of essential nutrients from the filter bed. The optimum moisture content does vary with different filter bed materials and their characteristics as well as different microbial populations, and can range from 30% to 110% [84–86].
2.6.8 Oxygen availability

Oxygen availability is essential for aerobic microbial degradation of pollutants in a biofilter. In order to provide sufficient oxygen for aerobic heterotrophic bacteria immobilized on the filter bed material a minimum of 5%-15% of oxygen in the inlet gas stream needs to be provided [40]. In general, O₂ is abundantly available in the inlet gas stream and is not considered to be a limiting factor. However, at high pollutant loads and a thick biofilm, O₂ could be limiting. A complete depletion of O₂ is undesirable since it can cause odours nuisance.

2.6.9 Pressure drop

During long-term operation pressure drop can be a very detrimental factor affecting overall biofiltration efficiency. The packing material and with that the particle size and shape, porosity and moisture content are factors with direct impact on the pressure drop along with biomass accumulation and superficial gas velocity. Changes in pressure drop can be linked to physical properties of the packing material [87]. With a gain in biomass accumulation the pressure drop increase is non-linear [88] and with increasing gas flow rate pressure drop increase approximately linearly [89]. For packed bed chemical reactors, the fluid characteristics and packing material properties have a major contribution to the pressure drop, which is generally caused by viscous and kinetic energy losses [87]. Therefore, to calculate the pressure drop in a biofilter, as a snap shot in time, the Ergun equation can be used.
Hee Wook Ryu [90] investigated the relationship between biomass, pressure drop and performance using benzene as a model VOC. Polyurethane (PU) foam cubes were used as packing material for the square acrylic column consisting of three layers each 0.3 x 0.3 x 0.3 m and a volume of 27 L each. The inlet concentration was maintained at 0.32 g m\(^{-3}\) and the space velocity (SV) at 200 h\(^{-1}\). Results indicated an increased pressure drop with increasing biomass concentration. In addition, numerical equations were obtained expressing the relationship between biofilter performance, biomass concentration and pressure drop. Roshani et al. [91] investigated strategies to overcome pressure drop built up in a hydrogen sulfide degrading biofilter. The two strategies used were leachate recycling and bed mixing. Whereas bed mixing did not show a significant effect likely due to the sulfur/sulfate accumulation. Opposing, leachate recycling had a double beneficial effect of improving environmental conditions for MO as well as for pressure drop build up.

Pressure drop as a function of moisture content at different bed heights and EBRT were investigated for a laboratory-scale wood chips packed column of a diameter of 0.1 m and a height of 1.5 m [87]. The author concluded 1) above a limiting moisture content of \(M_{\text{lim}} = 28.3\%\) the physical properties changed significantly, 2) at moisture contents < \(M_{\text{lim}}\) pressure drop increased marginally with increasing air flow, however for moisture contents > \(M_{\text{lim}}\) a more pronounced effect was observed, 3) specific surface area was reduced with increasing moisture content between 28.3% to 60% and 4) a developed Ergun –type equation can be used to predict pressure drop across the packed column with velocities starting from 0 m s\(^{-1}\) to 0.8 m s\(^{-1}\).
2.6.10 Empty bed residence time

The empty bed residence time (EBRT), as mentioned previously, relates the flow rate to the volume occupied by the filter bed material. It is a measure of how long the compound needs to travel through the packing material from the time it enters the packing material until it passed it. With an increase of EBRT, the contact time between the molecule and the packing material increases and hence the time available to diffuse from the gas phase to the interface of the liquid biofilm increases as well. Therefore, an enhanced removal efficiency (RE) can be observed [92,93]. EBRT evaluated in lab scale studies ranged from 45 s to 150 sec [94–96]. The applicability of a certain EBRT depends on the treated flow rate and possible size of the biofilter unit.

2.7 Microbiology of a biofilter

2.7.1 Microorganisms in a biofilter

Microorganisms and the development of a biofilm are essential for an effective VOC treatment in a biofilter. The microorganisms act as a biocatalyst for pollutant degradation [39]. The two mechanisms involved in the biodegradation of pollutants are either biotransformation into less complex metabolites or mineralization into non-toxic end products [39]. Usually a variety of microbes are present in biofilms, for example bacteria, fungi and algae [97]. The colony forming units (cfu) of bacteria in the biofilter, are in general in the range of $10^6$-$10^{10}$ cfu per g of bed material [98–100]. Despite the high number of bacteria in a biofilter, the proportion of bacteria degrading the pollutant of interest can be up to 30% of the total microbial population [101].
If a natural filter bed material is used such as compost, peat, etc. microorganisms are inherently and often abundantly present and may have the capability to degrade the gas phase pollutant. However, often the filter bed is inoculated with either a mixture of microbes or a specific microbe. For inoculation of a blend of microorganisms wastewater sludge is often used [58,102–104].

For recalcitrant pollutants, however, which may pose a higher chemical complexity in molecular structure, it may be necessary to use specific monocultures or microbial communities. The microbial communities along the filter bed height are likely to change, due to the differences in the environment. Concerning the concentration gradient along the filter media, high concentrations prevail near the inlet and with increasing distance the concentration decreases. Although this holds true only if a single pollutant is treated, since in a mixture of pollutant one substrate might be easier to degrade then others. Hence, the degradation of a pollutant might not occur or only occur very slowly. This gradient may alter microbial communities [105].

Ying et al. [102] showed the effect of different inlet loading rates on the change of bacterial communities in a biofilter. At lower concentrations or ILR (7.29 gs m$^{-3}$ h$^{-1}$) seven denaturing gradient gel electrophoresis (DGGE) bands were observed. With increasing dimethyl sulfide (DMS) loads however, the number of DGGE bands declined to 3 and rose to 8 as the concentration was increased again. In case with a mixture of pollutants, the more easily biodegradable compound is likely to biodegrade first and leaving other pollutants to biodegrade more slowly. This implies that along the filter bed different molecules will be bio-oxidized at different spatial compartments, which will lead to a different proliferation pattern of microbes [27]. Hence, the knowledge of microbial communities involved in
VOC biodegradation is an asset to optimize design and operation. As previously mentioned, different types of microbes are involved in biofiltration processes. Whereas bacteria are the most commonly studied microorganisms in biofiltration in recent years but the application of fungi for VOC treating is increasing [106–110]. In addition, the effectiveness of fungal-bacterial biofilters is being investigated [111].

A systematic comparison of fungal and/or bacterial biofiltration of toluene under steady and unsteady operations were completed in Cheng et al. [112]. Three biofilters, a fungal biofilter (F-BF), bacterial biofilter (B-BF) and fungal-bacterial biofilter (F&B-BF) were tested. For steady state operation the best RE of 100% were achieved by F&B-BF under EBRT of 96 s and ILR ranging from 11.25 g m\(^{-3}\) h\(^{-1}\) followed by the B-BF and F-BF with 82% and 85% respectively, under similar conditions. Next, inlet concentration fluctuations were tested at EBRT of 24 s and an inlet concentration ranging from 0.4 g m\(^{-3}\) – 1.0 g m\(^{-3}\). Under these conditions the F-BF reached the least RE > 30% followed by a little better performance of the B-BF and the F&B-BF.

### 2.7.2 Biofilm

When microorganisms find a desirable environment, they can avoid being carried away by attaching themselves to a surface. Those microorganisms then start to proliferate and excrete a polysaccharide like gel. An accumulation of microorganisms and gel substance as well as water lead to an embedding of microbes in a continuous layer and is referred to as a biofilm. A schematic of a biofilm is shown in Figure 2.2. Commonly, a biofilm is described as a thin layer at the interface between the solid and gas phase. Therefore, a biofilm is a crucial element of a biofilter carrying out the catabolic activity and bio-
transforming pollutants into CO₂, H₂O and heat. The growth of microbes and consequently the thickness of the biofilm are influenced by different factors such as type of pollutant, its flow rate through the biofilter, the filterbed material, diffusion behavior of the pollutant, availability of nutrients and the overall configuration of the treatment system. The biofilm thickness varies from a µm scale to an mm scale and as mentioned previously with increasing biofilm thickness the activity rises. However, if the thickness increases, diffusion-limitation gets more pronounced and nutrients potentially become a limiting factor.

Figure 2.2: Schematic of biofilm
2.7.3 Biodegradation and microbial growth kinetics

Knowledge of the kinetics of substrate biodegradation and microbial growth are important to understand and describe the behavior of a biofilter. The following section provides an overview of microbial growth kinetics and proposes different growth and substrate degradation models to describe a biofilter system.

2.7.3.1 Substrate degradation kinetics

A substrate degradation kinetic equation describes the depletion of a substrate in relationship to parameters such as growth rate and inhibitors, among others. Most commonly, Michaelis-Menten and Haldane kinetics are applied to describe microbial substrate degradation.

2.7.3.1.1 Michaelis-Menten kinetics

In order to mathematically describe the biodegradation of a substrate in a biofilter, which can be any VOC, Michaelis-Menten kinetics also known as enzyme kinetics are often used. These kinetic equations were developed based on experimental batch data where initial substrate $S_0$ and enzyme $E_0$ concentrations were known.

The general mechanism is described as an enzyme $E$ binds reversibly to a substrate $S$, with the rate constant $k_1$ (forward) and $k_2$ (backward) and consequently forms an enzyme-substrate complex $ES$. Afterwards the complex cleaves to the product $P$ and the enzyme with the rate constant $k_3$, which is illustrated as follows [113–115]:

\[ E + S \rightarrow ES \rightarrow E + P \]
\[ [E] + [S] \overset{k_1}{\underset{k_2}{\rightleftharpoons}} [ES] \rightarrow [E] + [P] \]

The associated rate equations are

\[ r_1 = k_1 [S][E] \]  \hspace{1cm} (2.12)
\[ r_2 = k_2 [ES] \]  \hspace{1cm} (2.13)
\[ r_3 = k_3 [ES] \]  \hspace{1cm} (2.14)

In order to develop a rate expression for the enzyme-catalyzed reaction the rapid-equilibrium assumption or the quasi-steady-state assumption can be used. For both the rate of production is described as [113–115]

\[ v = \frac{d[P]}{dt} = k_3 [ES] \]  \hspace{1cm} (2.15)

where \( v \) is the substrate consumption or product formation rate [g L\(^{-1}\) s\(^{-1}\)]. The rate of the ES complex is

\[ \frac{d[ES]}{dt} = k_1 [E][S] - k_2 [ES] - k_3 [ES] \]  \hspace{1cm} (2.16)

The conservation equation for the enzyme \( E \) is expressed as

\[ [E] = [E_0] - [ES] \]  \hspace{1cm} (2.17)

Where \( [ES] \) is the free enzyme concentration at time 0. This equations indicates that there will be a sudden decline in the substrate concentration \([ES]\) when the substrate is first brought into contact with the enzyme. The total enzyme \([Et]\) can be expressed as

\[ [Et] = [E] + [ES] \]  \hspace{1cm} (2.18)
[Et] is assumed to be constant. From here, one of the previously mentioned methods needs to be applied. In order to proceed the quasi-steady-state assumption is used. The assumption of that approach is to use a closed batch reactor where the initial substrate concentration greatly exceeds the initial concentration of the enzyme so that the concentration drop of [ES] discussed above is negligible. This means that the formation of the ES complex is very fast and stabilizes very quickly so that d[ES]/dt ≈ 0. This holds true only after a short transient phase, in which the concentration of ES increases and then remains approximately constant [115]. The pseudo-steady-state assumption is applied to the ES complex

\[ r_{\text{ES}} = r_1 - r_2 - r_3 = k_1 [S][E] - k_2 [ES] - k_3 [ES] = 0 \]  \hspace{1cm} (2.19)

hence,

\[ [\text{ES}] = \frac{k_1 [E][S]}{k_2 + k_3} \]  \hspace{1cm} (2.20)

The above equation is substituted into the total enzyme equation (2.18) and solved for E.

\[ [E] = \frac{[\text{Et}]}{1 + \frac{k_1}{k_2 + k_3} [S]} \]  \hspace{1cm} (2.21)

The overall rate of enzyme reaction equals \( r_3 \), since \( r_1 \) and \( r_2 \) are assumed to be sufficiently fast to reach an equilibrium between the free enzyme and the enzyme-substrate complex.

\[ r_3 = k_3 [\text{ES}] = k_3 \frac{k_1 [S][E]}{k_2 + k_3} \frac{k_1 [S]}{k_2 + k_3} \frac{[\text{Et}]}{1 + \frac{k_1}{k_2 + k_3} [S]} = k_3 \frac{k_1 [S][\text{Et}]}{k_2 + k_3 + k_1 [S]} = \frac{k_1 [S][\text{Et}]}{k_2 + k_3 + k_1 [S]} \]  \hspace{1cm} (2.22)

By defining the following variables

\[ V_{\text{max}} = k_3 [\text{Et}] \]  \hspace{1cm} (2.23)
\[ K_m = \frac{k_2 + k_3}{k_1} \]  \hspace{1cm} (2.24)

and substituting them into above equation the net rate of substrate degradation in a biofilm can be written as follows [113–115]:

\[ r = \frac{V_{\text{max}} [S]}{K_m + [S]} \]  \hspace{1cm} (2.25)

Equation 2.25 is referred to as the Michaelis-Menten equation and is widely used to describe biofiltration kinetics. \( V_{\text{max}} \) and \( K_m \) refer to the maximum reaction rate and the Michaelis-Menten constant, respectively. The units in context of biofiltration for \( V_{\text{max}} \) and \( r \) are usually mass of pollutant degraded per unit volume of biofilter per unit time and for \( K_m \) and \( S \) mass of pollutant per unit volume of biofilm. In a plot of reaction rate vs. substrate concentration \( K_m \) is the substrate concentration where the reaction rate is \( V_{\text{max}}/2 \). In case \( S \gg K_m \), \( K_m \) can be neglected and following pseudo zero order reaction rate is obtained [115]:

\[ r = V_{\text{max}} \]  \hspace{1cm} (2.26)

On the other hand, if \( S << K_m \) the reaction rate follows a first order rate [115]:

\[ r = \frac{V_{\text{max}}}{K_m} S \]  \hspace{1cm} (2.27)

### 2.7.3.1.2 Haldane kinetics

In cases of high substrate concentration some enzyme kinetics may be inhibited, which is known as substrate inhibition and can be described as follows [114]:
\[
\begin{align*}
[E] + [S] & \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [ES] \rightarrow [E] + [P] \\
+ & \\
S & \\
\overset{k_5}{\uparrow} & \overset{k_4}{\downarrow}
\end{align*}
\]

ES\textsubscript{2}

where, \(k_4\) is a forward reaction for building the ES\textsubscript{2} complex, and \(k_5\) the backward reaction.

The rate reactions describing the above system are as follows:

\[
\begin{align*}
r_1 &= k_1 [S][E] \quad \text{(2.28)} \\
r_2 &= k_2 [ES] \quad \text{(2.29)} \\
r_3 &= k_3 [ES] \quad \text{(2.30)} \\
r_4 &= k_4 [ES][S] \quad \text{(2.31)} \\
r_5 &= k_5 [ES2] \quad \text{(2.32)}
\end{align*}
\]

Next, two steady state approximations are made, one for ES and one for ES\textsubscript{2}.

\[
\begin{align*}
r[ES] &= r_1 - r_2 - r_3 + r_5 = k_1 [S][E] - k_2 [ES] - k_3 [ES] - k_4 [ES][S] + k_5 [ES2] = 0 \quad \text{(2.33)} \\
r[ES] &= r_4 - r_5 = k_4 [ES][S] - k_5 [ES2] = 0 \quad \text{(2.34)}
\end{align*}
\]

Adding both equations (2.33, 2.34) together leads to:

\[
\begin{align*}
r[ES] + r[ES2] &= r_1 - r_2 - r_3 = k_1 [S][E] - k_2 [ES] - k_3 [ES] = 0 \quad \text{(2.35)}
\end{align*}
\]

And consequently
\[ [ES] = \frac{k_1 [S][E]}{k_2 + k_3} \quad (2.36) \]

yield the same result as equation (2.20). Using the second pseudo-steady state approximation

\[ r[ES2] = r_4 - r_5 = k_4 [ES][S] - k_5 [ES2] = 0 \quad (2.37) \]

and substituting equation (2.36) and solving for ES, leads to

\[ [ES2] = \frac{k_4 [ES][S]}{k_5} = \frac{k_4 k_5 [ES][E][S]}{k_5 (k_2 + k_3)} = k_5 k_1 [S][E] \quad (2.38) \]

Now an enzyme balance is needed and can be described as follows:

\[ [Et] = [E] + [ES] + [ES2] \quad (2.39) \]

Substitution of equation (2.36) and equation (2.38) into equation (2.39) leads to:

\[ [Et] = [E] + \frac{k_1 [S][E]}{k_2 + k_3} + \frac{k_4 k_1 [E][S]^2}{k_5 (k_2 + k_3)} = [E] \left( 1 + \frac{k_1 [S]}{k_5 (k_2 + k_3)} + \frac{k_4 k_1 [S]^2}{k_5 (k_2 + k_3)} \right) \quad (2.40) \]

Solving for \([E]\) leads to:

\[ [E] = \frac{[Et]}{1 + \frac{k_1 [S]}{k_5 (k_2 + k_3)} + \frac{k_4 k_1 [S]^2}{k_5 (k_2 + k_3)}} \quad (2.41) \]

The overall reaction rate is \(r_3\) and can be expressed as:

\[ r_3 = k_3 [ES] = \frac{k_3 k_1 [E][S]}{k_2 + k_3} = \frac{k_3 k_1 [S][Et]}{(k_2 + k_3)} \left( 1 + \frac{k_1 [S]}{k_5 (k_2 + k_3)} + \frac{k_4 k_1 [S]^2}{k_5 (k_2 + k_3)} \right) \quad (2.42) \]

Dividing the numerator and denominator by \(k_1\), \(r_3\) can be written as:

\[ r_3 = \frac{k_1 [S][Et]}{k_1 + [S] + \frac{k_4 [S]^2}{k_1}} \quad (2.43) \]
Defining $V_{\text{max}}$, $K_m$ and $K_I$ and substituting into equation above, the overall substrate degradation rate in a biofilter can be written as [114]:

\[
V_{\text{max}} = k_3 [E_t] \quad \text{(2.44)}
\]

\[
K_m = \frac{k_2 + k_3}{k_1} \quad \text{(2.45)}
\]

\[
K_I = \frac{k_3}{k_4} \quad \text{(2.46)}
\]

\[
r = \frac{V_{\text{max}} [S]}{K_m + [S] + \frac{[S]^2}{K_I}} \quad \text{(2.47)}
\]

The equation above (2.47) is known as the Haldane equation, where $K_I$ represents the substrate inhibition constant [g pollutant m$^{-3}$ biofilm]. Besides the previously mentioned Monod kinetics, the Haldane kinetics is commonly to describe substrate biodegradation in the presence of substrate inhibition.

### 2.7.3.2 Microbial growth kinetics

Pollutant degradation is the result of microbial activity and hence the pollutant degradation rate is related to the microbial growth rate. The growth behavior of microorganisms can be divided into 5 phases [115]. In phase 1, called the lag phase, little to no increase of cell population occurs. During this phase, cells are adjusting to their new environment and synthesizing transport proteins for moving substrates into the cell, as well as synthesizing enzymes for utilizing substrate etc. and other processes. The duration of the lag phase can strongly vary and depends on the growth medium, the environmental conditions and on the type of microbe. When microorganisms start to grow, phase 2 starts, which refers to the
exponential growth phase, since the microbial growth rate is proportional to the cell concentrations. In this phase, cell proliferation are at the maximum because all necessary metabolic pathways are developed for utilizing substrate as a result of phase 1. After maximum growth and eventually lack of nutrients, built up of toxic products or other limitations the net growth rate drops, growth will stagnate and the stationary phase starts. However, in fermentation processes many products, such as penicillin are produced in this phase. The stationary phase is followed by phase 4, the death phase, in which a decrease of living cells occurs. In this phase the decay rate is greater than the growth rate. This may be a result of the toxic by-products, harsh environment and/or depletion of nutrients.

Since in the exponential phase microbial growth rate is proportional to the cell concentration, it is used to determine the growth rate of microbial population and is described as follows [115]:

\[ \frac{dx}{dt} = \mu_{net} X \]  \hspace{1cm} (2.48)

where \( X, \mu_{net} \) and \( t \) express the biomass concentration [g L\(^{-1}\)], the coefficient of proportionality or net specific growth rate [h\(^{-1}\)] and the time [h]. The net specific growth rate is the difference between the gross specific growth rate and rate of cell mass decay due to cell death or endogenous metabolism. The net specific growth rate can be described as follows:

\[ \mu_{net} = \mu - a \]  \hspace{1cm} (2.49)

where \( \mu \) refers to the gross specific growth rate [h\(^{-1}\)] and \( a \) expresses the cell mass decay [h\(^{-1}\)]. The Yield is specified by the ratio of the growth rate of microbial population to the rate of substrate biodegradation as follows:
2.7.3.2.1 Monod kinetics

The relationship of the specific growth rate to substrate concentration often assumes the form of a saturation kinetics. These kinetics are similar to the Michaelis-Menten kinetics for an enzyme reaction. This specific growth rate of microbial population depends on substrate concentration and can be described by the Monod equation. For VOC biofiltration the Monod equation has been used most commonly to describe microbial growth. The applied Monod equation is expressed as follows [113–115]:

\[ \mu = \frac{\mu_{\text{max}} S}{K_s + S} \]

(2.51)

where \( \mu_{\text{max}} \) is the maximum specific growth rate and \( K_s \) the saturation constant or half velocity constant and is equal to the concentration of the rate-limiting substrate when the specific rate of growth is equal to one-half of the maximum. It is assumed that \( K_s \) and \( K_m \) are the same in equation 2.48 The yield can be described as:

\[ Y = \frac{\mu_{\text{max}} X}{V_{\text{max}}} \]

(2.52)

2.7.3.2.2 Haldane kinetics

The substrate itself potentially can act as an inhibitor and this is known as substrate inhibition or Haldane kinetics. Haldane kinetics occur when the reactant is also an uncompetitive inhibitor. In biofiltration it has been found that substrate inhibition affects the system. Haldane kinetics can be described with the following equation [114,115].
\[ \mu = \frac{\mu_{\text{max}} S}{K_m + S + \frac{S^2}{K_i}} \]  

(2.53)

Where \( K_i \) is the inhibition constant and \( K_s \) is here equal to \( K_m \). At low substrate concentration \( K_m >> (S + S^2/K_i) \) approximates

\[ \mu \approx \frac{\mu_{\text{max}} S}{K_m} \]  

(2.54)

and at high substrate concentrations \( (S^2/K_i) >> (K_m + S) \) yields

\[ \mu \approx \frac{\mu_{\text{max}} K_i}{S} \]  

(2.55)

Hence, the resulting kinetic expression has a reaction order of -1.

2.8 Biofilter models

In a biofilter model, microbial growth kinetics, substrate biodegradation, mass transfer and reaction engineering are linked. The development of a biofilter model is rather difficult, since biological systems are quite complex. Parameters which describe the filter bed medium are difficult to obtain, e.g. biofilm thickness, diffusion coefficient of analyte across the biofilm, etc. Therefore, reasonable assumptions have to be made and are mentioned as follows [116]. Most biofilter models assume that air flow can be modeled as plug flow. A common attempt to determine concentration on the surface of the biofilm is by using Henry’s Law equilibrium with the concentration of the contaminant in the bulk gas phase. The liquid phase within the biofilter is presumed to be stagnant, and therefore, molecular diffusion is the only transport mechanism. Diffusion of the substrate into the biofilm is assumed to follow Fick’s Law. Biodegradation rates of MO are fundamental for
biofiltration and are most commonly assumed to follow Monod kinetics for microbial growth. Also, models are developed for steady-state and transient state behavior of bioreactors. A few notable developed models are by Ottengraf and Van Den Oever [117], Shareefdeen et al. [118], Deshusses et al. [119,120] and Hodge and Devinny [121].

The proposed model for toluene, butylacetate, ethylacetate and butanol degradation by Ottengraf and Van Den Oever [117] was one of the first biofilter models. It assumes 1) the resistance at the interface between biolayer and the gas phase is negligible, hence Henry’s law is applicable, 2) nutrient transport in the biolayer occurs by diffusion, 3) compared to the diameter of the packing particle, the thickness of the biofilm is negligible, 4) substrate degradation kinetics follows a zero-order rate, and 5) the gas flow can be considered as plug flow. This basic biofilter model does not account for substrate inhibition effect or biomass growth, and it is difficult to determine the rate limiting factor.

Shareefdeen et al. [118] developed the first steady state biofilter model to describe methanol vapor degradation, and one assumption they made was that oxygen and the substrate were reaction rate limiting components. Its advantage is e.g. that it includes substrate inhibition, whereas a constant biofilm density is considered. In addition, the model includes diffusion limitation in a biofilter.

The models developed by Deshusses et al. [119,120] includes pollutant interaction and considered biodegradation of methyl ethyl ketone and methyl isobutyl ketone in a downward flow biofilter. One of the advantages is that it incorporates substrate inhibition and substrate interaction. It also accounts for diffusion limitation by dividing the biofilm into four well-mixed layers, and assume first-order mass transfer between the layers. This is a dynamic model.
The Hodge and Devinny et al. [121] model simplified the Shareefdenn and Baltzis model and described the biofilter as a two-phase system; the air phase and the solids/water phase. Limitation of that proposed model are e.g. that a growth model was not considered and substrate inhibition effect was neglected.

Li and De Visscher [122] developed a toluene biofilter model that combines Haldane kinetics with the Verhulst equation for microbial growth. The main advantage is, the description of changes in activity as a result of changing concentration or flow rate. Haque [123] replaced the Verhulst portion of the model of Li and De Visscher by a nitrogen dynamics and limitation model. This model includes toluene limitation but not oxygen limitation. The assumption of a variable biofilm thickness, allows the flexibility in deciding whether or not the diffusion limitation should be considered on the calculated biofilm thickness, which is a significant advantage of the model.
3 Biological treatment of waste gases contaminated with benzene, toluene, ethylbenzene or m-xylene

3.1 Abstract

*Nocardia* sp. previously isolated from compost is evaluated in terms of its degradation capability to remove a single pollutant benzene, toluene, ethylbenzene or m-xylene at empty-bed residence times of 1.5 and 2.5 min. Laboratory scale biofilters packed with compost and straw (vol% 80/20), inoculated with *Nocardia* sp. as hydrocarbon degrader and 2 unknown non-hydrocarbon degrading bacteria at an average inlet concentration of 0.050 g m$^{-3}$, 0.600 g m$^{-3}$ and 1.5 g m$^{-3}$ of single hydrocarbon were continuously operated. The performance analysis indicated RE > 95% at low inlet concentrations and EBRT of 2.5 min for toluene, ethylbenzene and m-xylene. With increasing inlet concentration RE declined and therefore, it is suggested that the inoculant is more efficient at low inlet concentration then more commonly used organisms. In addition, the determined kinetic parameters revealed with a marked deviation from literature results with more commonly studied organisms. At low inlet concentrations, the operated biofilter systems achieved or exceeded efficiencies found in other studies.
3.2 Introduction

Anthropogenically released gas emissions can contain a variety of pollutants such as volatile organic compounds (VOCs). BTEX are a group of aromatic hydrocarbons comprising benzene, toluene, ethylbenzene and xylenes. The chemical, oil and gas, pharmaceutical and pulp and paper industry are potential sources of these air pollutions. Under long-term exposure, BTEX adversely affects the nervous, digestive, kidney and respiratory systems [29]. All four compounds are listed as Hazardous Air Pollutants under the United States Environmental Protection Agency [124], and benzene is considered a carcinogen for humans [13].

A variety of conventional physico-chemical air pollution control methods to reduce the VOC and consequently BTEX air emissions are available, for instance catalytic oxidation, incineration and adsorption. However, higher operational and maintenance costs or production of unwanted or even toxic byproducts are adjunct drawbacks. Biological methods such as biofiltration have been found to be a sustainable, effective and efficient alternative. Biofiltration is a well-established method for odour and VOC treatment at low concentration (< 5 g m⁻³). Microorganisms biodegrade the desired compound and produce CO₂, H₂O and heat. In the metabolic pathway for degrading BTEX, two enzymes, 1) dioxygenase and 2) monooxygenase, attack the substituent group on the aromatic ring (for toluene, ethylbenzene and xylenes) or the aromatic ring (for benzene) [125].

VOC and BTEX biofiltration have been studied for more than a decade, covering the evaluation of process parameters such as inlet loading, moisture content, mesophilic and thermophilic temperature conditions, filter bed material and most recently the application of microbial fuel cells [53,71,105,126,127]. Either an unknown mixture of microorganisms
or a specifically chosen microorganism was used. A common method to inoculate a biofilter with an unknown mixture of microorganisms is to use sludge from a wastewater treatment plant. In this case, microorganisms, which are favored by the environmental conditions, proliferate and consequently a natural microbial hierarchy occurs [128,129]. The behavior of a biofilter can change depending on the single bacterium, fungus or mixture of bacteria or/and fungi. Single bacteria as well as consortia of bacteria or fungi have been studied in terms of their application and efficiencies [130–133]. If a single bacterium is used, the biofilter needs to be kept sterile in order to avoid an interaction with other microorganisms and a potential shift in efficiency. Such an interaction might be beneficial or unfavorable, but the effect is difficult to predict. For this reason, an unwanted introduction of a microbe, for example due to the inlet gas stream, nutrient solution etc., needs to be prevented, or the used filterbed material needs to be sterilized, which is associated with additional costs.

Hence, in this study a BTEX degrading bacterium, Nocardia sp., a bacterium found to degrade toluene in pure culture in a previous study [134], was evaluated in terms of its capabilities to treat BTEX in the presence of two other, non BTEX degrading bacteria.

3.3 Materials and methods

3.3.1 Microorganism and filter media

For the filter bed a mixture of sterilized compost and straw (vol% 80/20) was used. Compost and straw are natural products, and are readily available. Straw is an agricultural by- or waste product. In Table 3.1, the filter bed analysis is shown and was conducted by AGAT Laboratories. Benzene, toluene, ethylbenzene, m-xylene and solids for the
cultivation medium were obtained from Sigma Aldrich. An adapted liquid BH-medium [123] was used as a growth medium and contained 1 g L\(^{-1}\) KH\(_2\)PO\(_4\), 1 g L\(^{-1}\) Na\(_2\)HPO\(_4\), 0.5 g L\(^{-1}\) NH\(_4\)NO\(_3\), 0.002 g L\(^{-1}\) FeCl\(_3\), 0.002 g L\(^{-1}\) MnSO\(_4\) 2H\(_2\)O, 0.2 g L\(^{-1}\) MgSO\(_4\) 7H\(_2\)O and 0.02 g L\(^{-1}\) CaCl \(_2\)H\(_2\)O.

As a single carbon source, benzene, toluene, ethylbenzene and \(m\)-xylene were used, respectively. Serum bottles sealed with a butyl rubber septum and aluminum crimp seal and containing liquid sterilized cultivation medium were used to cultivate the microbes. *Nocardia* sp., the indigenous bacterium in the pure culture, were blended with two non BTEX degrading bacteria. An air stream was piped into the serum bottle in order to introduce bacteria from the ambient air into the inoculant. Plating of cells was conducted and standard procedures were followed [135]. Cell plating indicated one microbe that is able to degrade toluene, which is *Nocardia* sp., since this is the indigenous bacterium and known to degrade toluene. Two other bacteria were found in the inoculant and a degradation test showed no ability to degrade toluene. In order to conduct the degradation test, only toluene was provided as a carbon source. Hence, it is assumed that *Nocardia* sp. is the only BTEX degrading microbe in the inoculant. Further characterizations were not conducted. Cultivation of the inoculant was carried out under ambient temperature (21-22°C).
Table 3.1: Filterbed properties used in this study

<table>
<thead>
<tr>
<th>Filterbed properties</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Nitrogen</td>
<td>15900</td>
<td>mg/kg</td>
</tr>
<tr>
<td>NO$_3$ - N and NO$_2$-N</td>
<td>916</td>
<td>mg/kg</td>
</tr>
<tr>
<td>Organic Matter</td>
<td>29.50</td>
<td>%</td>
</tr>
<tr>
<td>PO$_4$-P</td>
<td>194</td>
<td>mg/kg</td>
</tr>
<tr>
<td>pH</td>
<td>7.30</td>
<td></td>
</tr>
<tr>
<td>Particle Size Distribution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2000-50 μm)</td>
<td>44</td>
<td>%</td>
</tr>
<tr>
<td>(50-2 μm)</td>
<td>37</td>
<td>%</td>
</tr>
<tr>
<td>(&lt;2 μm)</td>
<td>19</td>
<td>%</td>
</tr>
</tbody>
</table>

3.3.2 Biofilter set-up and experimental conditions

The BTEX biodegradation was carried out in a sterilized lab-scale biofilter with an inoculation volume of 100 mL and an optical density (OD$_{650}$) of around 0.2 for each inoculant. For the ethylbenzene column, cultivation of microorganisms was conducted by using ethylbenzene as a carbon source. For the other columns, toluene was used as a carbon source during cultivation. All four columns were made of polycarbonate with a total height of 63 cm and an internal diameter of 10 cm. At the bottom, glass beads at a height of 5 cm and a 1 cm perforated Plexiglass plate were used to evenly distribute the inlet gas stream. Sampling ports to measure gas samples were centered at the top and bottom of the column and sealed with GC septa (0.95 cm diameter). The biofilters were filled up with the filter media to a height of 25 cm and placed in a fume hood under an ambient temperature of 21-22°C. A schematic of the experimental set-up is shown in Figure 3.1. An air pump (pond master Ap-40) was used to generate one air stream, which was split and sent through two gas washing bottles, one filled with tap water and one filled with the contaminant.
Subsequently, the two gas flows were combined and mixed in an empty gas-washing bottle before being introduced into the biofilter. The flow was controlled by two rotameters (Cole-Parmer) located after the gas flow split, and total flow rate was measured (TI-400) prior to the sample port and inlet. The operational parameters are summarized in Table 3.2 and Table 3.3.

Figure 3.1: Schematic of a biofilter setup: 1) air pump, 2) rotameters, 3) sterilized water, 4) contaminant, 5) mixing vessel, 6) flow meter and 7) biofilter
3.3.3 Biofilter performance parameters

The performance of a biofilter is commonly characterized by the empty-bed residence time (EBRT), inlet loading rate (ILR), removal efficiency (RE) and elimination capacity (EC). They are defined as follows:

\[
\text{EBRT} = \frac{V}{Q} \quad (2.1)
\]

\[
\text{ILR} = \frac{Q}{V} \cdot \frac{C_i}{V} \quad (2.5)
\]

\[
\text{RE} = \frac{C_i - C_o}{C_i} \times 100 \quad (2.6)
\]

\[
\text{EC} = \frac{Q(C_i - C_o)}{V} \quad (2.7)
\]

where \(C_i\), \(C_o\), \(Q\), and \(V\) represent the inlet concentration [g m\(^{-3}\)], outlet concentration [g m\(^{-3}\)], flow rate [m\(^3\) h\(^{-1}\)] and volume [m\(^3\)], respectively.

3.3.4 Biofilter operation

An air stream contaminated with single compounds of benzene, toluene, ethylbenzene or \(m\)-xylene was treated under different operational conditions. Two different EBRT (1.5 min and 2.5 min) were examined and for each EBRT a stepwise increase of the inlet concentration was conducted. The average inlet concentration and corresponding EBRT are shown in Table 3.2 and Table 3.3. An adsorption test for each compound was carried out and did not indicate any adsorption due to the sterilized packing material nor Plexiglas. Results are shown in Appendix A.
3.3.5 Kinetic batch tests

The kinetic characterization of the biofilter inoculant (bacteria) was performed under sterile conditions in batch tests. The batch tests were conducted in 160 mL serum bottles sealed with butyl rubber septa and aluminum crimp seals. Each serum bottle was filled with 100 mL of the adapted liquid BH-medium ($\text{OD}_{650}$ were around 0.1), which were previously adapted to the used contaminant. The initial liquid volume of benzene, toluene, ethylbenzene and $m$-xylene added to the serum bottles was 1 µL. The biodegradation tests were carried out at room temperature (21-22˚C) and the medium was continuously mixed to avoid mass transfer limitation. Duplicates of each test were performed and the average value of the results are presented further down. Control tests were conducted without bacteria and the result indicated no adsorption due to the liquid phase. Figures are presented in Appendix B. The gas phase concentration was measured; however, kinetic parameters are represented for the liquid phase (e.g. $K_m$ as liquid phase concentration). Measurements were taken after injecting the liquid contaminate and for analyzing the experimental data, simulation were conducted following the approach outlined in [136]. Assuming that Henry’s law applies, the differential equation to be integrated numerically is:

$$
\left(\frac{V_L}{H} + V_G\right) \frac{dC_G}{dt} = -m \ r
$$

(3.1)

where $V_L$ is the volume of the liquid phase [m$^3$], $V_G$ is the volume of the gas phase [m$^3$], $H$ is the Henry constant of the BTEX [ ], $C_G$ is the gas phase concentration of toluene [g m$^{-3}$], $t$ is the time [h], $m$ the biomass [g], and $r$ is the BTEX degradation rate [g m$^{-3}$ h$^{-1}$]. To account for lag phase, the reaction rate $r$ in eq. (3.5) was replaced by $\alpha x r$, where $\alpha$ is given by:
\[
\alpha = \frac{q_0}{q_0 + e^{(-\upsilon)}} 
\]  

(3.2)

The reaction rate \( r \) is given by equation 3.3 (essentially the same as equation 2.47):

\[
\begin{align*}
\dot{r} &= \frac{V_{\text{max}}}{K_m + C_L} \frac{C_L}{K_l + C_L} \\
\text{where } C_L &= C_G/H.
\end{align*}
\]  

(3.3)

The numerical integration was carried out by Euler’s method in Excel, and the parameters \( V_{\text{max}}, K_m, \) and \( K_l \) as well as \( q_0 \) and \( \upsilon \) were determined by nonlinear least squares, using the solver function in Excel.

### 3.3.6 Analytical methods

A SGE 250 µL gastight syringe was used to draw and inject 200 µL of gas sample from the gas sampling ports and into the analyzer. The gas samples were analyzed with a gas chromatograph (GC-2014, Shimadzu) equipped with an FID and Rtx®-Wax capillary column (30 m x 0.53 mm x 1 µm). The injector and detector temperatures were set at 250°C. The oven temperature for benzene, toluene, ethylbenzene and \( m \)-xylene were 70°C, 80°C, 90°C and 100°C, respectively. Helium was used as a carrier gas.
3.4 Results and discussion

3.4.1 BTEX removal efficiency

3.4.1.1 Phase 1 – EBRT – 1.5 min

The initial concentration for startup was about 0.050 g m\(^{-3}\) for each biofilter, as shown in Table 3.2. REs, with Nocardia sp. as degrading bacterium, of 96.85% and 93.42% were achieved for ethylbenzene and toluene at inlet concentration of around 0.050 g m\(^{-3}\), which correspond to an EC of 1.70 and 1.91 g m\(^{-3}\) h\(^{-1}\). Seven days and 14 days after startup, ethylbenzene and toluene degradation reached a maximum, as shown in Figure 3.2. Similar results were reported in [137] for an EBRT of 58.8 s and an inlet toluene concentration ranging from 0.04 to 0.2 g m\(^{-3}\). However, a mixed microbial population was used as inoculant, whereas in present study only Nocardia sp. under the presence of two other bacteria achieved such high RE. Here, after 20 days of acclimatization, the achieved RE was > 96%. The shorter achieved acclimatization time might be due to the higher EBRT. The achieved average toluene EC of 19.2 g m\(^{-3}\) h\(^{-1}\) for an EBRT of 1.5 min and an average ILR of 26.9 g m\(^{-3}\) h\(^{-1}\) are higher in the present study as compared to the results of [138], where for an EBRT of 1.5 min and an average ILR of 22.4 g m\(^{-3}\) h\(^{-1}\) the achieved average EC was 12 g m\(^{-3}\) h\(^{-1}\). Consequently, the measured average EC was higher in this study although the biofilters were exposed to higher ILR. In this study, biodegradation of ethylbenzene with an EBRT of 1.5 min, and an average ILR of 34.1 g m\(^{-3}\) h\(^{-1}\) (corresponding to an average of 0.886 g m\(^{-3}\) ethylbenzene), an average EC of 17.8 g m\(^{-3}\) h\(^{-1}\) was achieved. Compared to [138] an average EC of 12.9 g m\(^{-3}\) h\(^{-1}\) was achieved under the same EBRT of 1.5 min and an average IL of 27.4 g m\(^{-3}\) h\(^{-1}\). Consequently, under the same EBRT and higher ILR the achieved EC of ethylbenzene in this study was higher.
Hence, the investigated inoculant (*Nocardia* sp. and non-degrading bacteria) are able to achieve higher efficiencies compared to an inoculant obtained from a wastewater treatment plant. In present study, benzene and *m*-xylene degradation were not observed after 6 and 11 days, respectively. Therefore, the inlet concentration was increased to 0.110 and 0.140 g m$^{-3}$, respectively, and a maximum RE of 100% for benzene and 87.15% for *m*-xylene were obtained after 15 days. The reason for such a behavior could be the lack of sufficient carbon source to trigger microbial growth at such low concentrations. When the specific growth rate does not exceed the endogenous respiration rate, there is no net growth of bacteria.
<table>
<thead>
<tr>
<th>Phase 1 - EBRT 1.5 min</th>
<th>Average inlet</th>
<th>max EC</th>
<th>max RE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[g m⁻³]</td>
<td>[g m⁻³ h⁻¹]</td>
<td>[%]</td>
</tr>
<tr>
<td>toluene</td>
<td>0.05</td>
<td>1.70</td>
<td>93.42</td>
</tr>
<tr>
<td>ethylbenzene</td>
<td>0.05</td>
<td>1.91</td>
<td>96.85</td>
</tr>
<tr>
<td>benzene</td>
<td>0.05</td>
<td>0.01</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>0.11</td>
<td>5.07</td>
<td>100.00</td>
</tr>
<tr>
<td>m-xylene</td>
<td>0.08</td>
<td>-0.04</td>
<td>-1.03</td>
</tr>
<tr>
<td></td>
<td>0.14</td>
<td>6.18</td>
<td>87.15</td>
</tr>
<tr>
<td>toluene</td>
<td>0.68</td>
<td>22.70</td>
<td>71.08</td>
</tr>
<tr>
<td>ethylbenzene</td>
<td>0.85</td>
<td>22.95</td>
<td>63.74</td>
</tr>
<tr>
<td>benzene</td>
<td>0.67</td>
<td>24.89</td>
<td>53.76</td>
</tr>
<tr>
<td>m-xylene</td>
<td>0.93</td>
<td>12.30</td>
<td>32.98</td>
</tr>
<tr>
<td>toluene</td>
<td>1.44</td>
<td>15.96</td>
<td>26.77</td>
</tr>
<tr>
<td>ethylbenzene</td>
<td>1.65</td>
<td>12.55</td>
<td>17.73</td>
</tr>
<tr>
<td>benzene</td>
<td>1.61</td>
<td>3.47</td>
<td>6.29</td>
</tr>
<tr>
<td>m-xylene</td>
<td>1.42</td>
<td>22.76</td>
<td>36.03</td>
</tr>
</tbody>
</table>
After increasing the inlet concentration RE dropped and degradation efficiency was highest for toluene, followed by ethylbenzene, benzene and m-xylene. A similar behavior was observed as the inlet concentration was adjusted to its highest level. Such an increase of RE with increasing inlet concentration is different compared to results found in literature, where a decrease is usually observed. A possible explanation could be, that microbes grow and hence activity increases, which lead to a higher reaction rate and RE. This can be seen in Figure 3.5 d. Here the reaction rate as a function of concentration is plotted and a linear increase of reaction rate with increasing concentration can be observed. Maximum elimination capacities were obtained in the medium concentration range and reached 24.89, 22.95 and 22.70 g m\(^{-3}\) h\(^{-1}\) for benzene, ethylbenzene and toluene, respectively.
(a) and (b) show the removal efficiency and concentration changes over time. The graphs indicate the changes in concentration [g m⁻³] and removal efficiency [%] with time [day] for both inlet and outlet samples. The plots highlight the efficiency and concentration trends over the specified time periods.
Figure 3.2: RE, inlet and outlet concentration of Phase 1 - EBRT 1.5 min, a) benzene, b) toluene, c) ethylbenzene, d) m-xylene
3.4.1.2 Phase 2 - EBRT 2.5 min

In Phase 2 the average EBRT was adjusted to 2.5 min and the inlet concentrations were reduced to an average of 0.04 g m\(^{-3}\) for toluene and ethylbenzene, as shown in Table 3.3.

Table 3.3: Operational parameters for Phase 2 - EBRT 2.5 min

<table>
<thead>
<tr>
<th></th>
<th>Average inlet [g m(^{-3})]</th>
<th>max EC [g m(^{-3}) h(^{-1})]</th>
<th>max RE [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase 2 - EBRT 2.5 min</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toluene</td>
<td>0.04</td>
<td>1.15</td>
<td>100</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>0.04</td>
<td>1.18</td>
<td>96.75</td>
</tr>
<tr>
<td>Benzene</td>
<td>0.11</td>
<td>3.56</td>
<td>95.20</td>
</tr>
<tr>
<td>m-Xylene</td>
<td>0.10</td>
<td>2.18</td>
<td>96.15</td>
</tr>
<tr>
<td>Toluene</td>
<td>0.63</td>
<td>9.45</td>
<td>68.00</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>0.88</td>
<td>17.22</td>
<td>66.50</td>
</tr>
<tr>
<td>Benzene</td>
<td>0.78</td>
<td>10.59</td>
<td>55.19</td>
</tr>
<tr>
<td>m-Xylene</td>
<td>0.78</td>
<td>8.19</td>
<td>43.58</td>
</tr>
<tr>
<td>Toluene</td>
<td>1.50</td>
<td>9.20</td>
<td>28.12</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>1.69</td>
<td>8.64</td>
<td>19.34</td>
</tr>
<tr>
<td>Benzene</td>
<td>1.77</td>
<td>5.33</td>
<td>11.55</td>
</tr>
<tr>
<td>m-Xylene</td>
<td>1.45</td>
<td>4.27</td>
<td>11.85</td>
</tr>
</tbody>
</table>
The starting concentrations for benzene and m-xylene were set to 0.11 and 0.10 g m\(^{-3}\) respectively, since in phase 1 no degradation was observed for a lower concentration, shown in Figure 3.3. The maximum toluene RE increased to 100% and an average EC of 1.15 g m\(^{-3}\) h was achieved for toluene biofiltration. The increase of EBRT and change of toluene inlet concentration led to an RE > 90% at day one of phase 2. In Rene et al. [139] acclimatization of a compost biofilter treating toluene was conducted at an EBRT of 2.45 min and an inlet concentration in the range of 0.04-0.07 g m\(^{-3}\). The RE was higher than 90%, which is a similar result compared to present study. Here, the investigated microbe *Nocardia* sp. are able to achieve higher RE than a mixture of microbes use in the other study. At an average ethylbenzene inlet concentration of 0.04 g m\(^{-3}\) h\(^{-1}\) (ILR of 1.21 g m\(^{-3}\) h\(^{-1}\)) and an EBRT of 1.5 min an RE of 96.75% or an EC of 1.18 g m\(^{-3}\) h\(^{-1}\) were achieved. Similar results were shown in [74], where an RE of > 95% was found at an EBRT of around 2 min and an ethylbenzene inlet concentration range from 0.3-1 g m\(^{-3}\). For an average benzene inlet concentration of 0.78 g m\(^{-3}\) the maximum RE was 55%. With further increase of the inlet concentration, the RE declined to < 11.5%. Although low efficiencies at high and medium concentration, RE > 95% were achieved for concentration around 0.11 g m\(^{-3}\). This indicates that *Nocardia* sp. biofilters are an effective application for low benzene concentrations.

A stepwise increase to the middle range resulted in an increase of RE for ethylbenzene, and *m*-xylene. The highest increase was observed for m-xylene to 43.58% an increase of > 10% compared to phase 1. In the medium inlet concentration range toluene RE did not increase from phase 1 to phase 2. This does not necessarily indicate a lower average efficiency, since the maximum RE was measured at a single day and therefore only
represents a snap-shot in time. The \textit{m}-xylene RE achieved for an inlet concentration of 1.45 g m$^{-3}$ was 11.85\%. This result is lower than the RE observed in phase 1 with a similar inlet concentration of 1.42 g m$^{-3}$. RE is expected to increase with increasing EBRT and constant inlet concentration, but this behavior was not observed. A reason could be the high biomass accumulation in phase one, which led to higher RE, followed by an inhibition in phase 2.
Figure 3.3: RE, inlet and outlet concentration for Phase 2 - EBRT 2.5 min, a) benzene, b) toluene, c) ethylbenzene, d) m-xylene
3.4.2 Kinetics and parameter estimation

In order to determine the parameters for the Haldane-kinetics, equation 3.4 (essentially the same equation as 2.47) was applied to study single substrate biodegradation.

\[-r' = \frac{V_{\text{max}} c}{K_m + c + \frac{c^2}{K_I}}\]  \hspace{1cm} (3.4)

where \( r \) represents the reaction rate [g m\(^{-3}\) h\(^{-1}\)], \( c \) is the concentration of contaminant [g m\(^{-3}\)], \( V_{\text{max}} \) is the maximum reaction rate [g m\(^{-3}\) h\(^{-1}\)], \( K_m \) expresses the half-saturation constant [g m\(^{-3}\)] and \( K_I \) is the inhibition constant [g m\(^{-3}\)]. In order to simulate the lag-phase the method of [136] was applied, and was previously explained. The computed average liquid phase parameters are represented in Table 3.4 and corresponding figures are depicted in Figure 3.4. In addition, a plot of reaction rate as a function of concentration is depicted in Figure 3.5. In some trials, no lag phase can be observed which might be due to the enrichment of culture in respective substrates before conducting the batch tests.

Comparing the estimated kinetic parameters to literature values, shown in Table 3.5, it is observed that the parameters are generally outside the range found in other studies [140–142]. \( K_m \) and \( K_I \) of the experiments are significantly smaller than those found in literature. Where a low \( K_m \) value corresponds to a higher affinity of the compound to the microbes, a low \( K_I \) value is representative for a high sensitivity to substrate inhibition [141]. The initial concentration of the substrate during the experiments is < 3.5 g m\(^{-3}\) for all trials. The \( V_{\text{max}} \) of toluene is consistent with other studies. However, the maximum reaction rates for benzene and ethylbenzene are higher. The parameters indicate that \textit{Nocardi} sp. is a more oligotrophic degrader than the organisms usually found in BTEX biofilters (e.g. \textit{Pseudomonas}). This is in general agreement with the results of the biofilter texts.
discussed above. Due to the lack of kinetic parameters for \textit{m}-xylene in the literature, no comparison could be made. However, a higher value of $K_m$ (191.40 g m$^{-3}$) indicates a lower affinity of the microbes to \textit{m}-xylene compared to those of toluene, benzene and ethylbenzene in this study. In addition, a $K_I$ of 150.56 g m$^{-3}$ is indicative of a higher degree of persistence against substrate inhibition. However, with these values Haldane kinetics essentially follows a first-order kinetics ($V_{\text{max}}/K_m C$), since the concentration in the batch tests were < 3 g m$^{-3}$. In addition, the observed differences between obtained results and literature values might be based on the different microbes which were used for conducting the tests.
Concentration [g m\(^{-3}\)] vs Time [hours]

ai) Experiment vs Num integration

aii) Experiment vs Num integration
Figure 3.4: Batch test experiments and model prediction for a) toluene, b) ethylbenzene, c) benzene and d) m-xylene
Reaction rate \( \text{[g m}^{-3}\text{h}^{-1}] \) vs. Concentration \( \text{[g m}^{-3}] \)

iii)
Reaction rate $[\text{g m}^{-3} \text{h}^{-1}]$ vs Concentration $[\text{g m}^{-3}]$

**bii)**
Reaction rate $[\text{g m}^{-3} \text{h}^{-1}]$

Concentration $[\text{g m}^{-3}]$

\(\text{ci)}\)

\(\text{cii)}\)
Figure 3.5: Prediction of reaction rate as a function of concentration for a) toluene, b) ethylbenzene, c) benzene and d) m-xylene
Table 3.4: Average kinetic parameters of the liquid phase determined by using Haldane kinetic

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_I$</th>
<th>$V_{\text{max}}$</th>
<th>$K_m$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene</td>
<td>2.47</td>
<td>0.61</td>
<td>0.050</td>
<td>0.94</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>9.16</td>
<td>1.52</td>
<td>0.087</td>
<td>0.93</td>
</tr>
<tr>
<td>Benzene</td>
<td>7.90</td>
<td>1.27</td>
<td>0.086</td>
<td>0.94</td>
</tr>
<tr>
<td>$m$-Xylene</td>
<td>150</td>
<td>0.58</td>
<td>191</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Table 3.5: Literature values of kinetic parameters

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_I$</th>
<th>$\mu_{\text{max}}$</th>
<th>$K_m$</th>
<th>Yield</th>
<th>$V_{\text{max}}$</th>
<th>Microbes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene</td>
<td>42.78</td>
<td>0.42</td>
<td>3.98</td>
<td>0.93</td>
<td>0.45</td>
<td>P. putida 54G</td>
<td>[143]</td>
</tr>
<tr>
<td></td>
<td>78.94</td>
<td>0.86</td>
<td>11.03</td>
<td>0.71</td>
<td>1.21</td>
<td>consortia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>44.43</td>
<td>0.72</td>
<td>15.07</td>
<td>0.64</td>
<td>1.13</td>
<td>P. putida</td>
<td>[144]</td>
</tr>
<tr>
<td></td>
<td>45.00</td>
<td>0.46</td>
<td>0.65</td>
<td>0.95</td>
<td>0.48</td>
<td>P. putida F1</td>
<td>[144]</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>20.00</td>
<td>0.26</td>
<td>1.50</td>
<td>0.40</td>
<td>0.65</td>
<td>P. putida F1</td>
<td>[145]</td>
</tr>
<tr>
<td></td>
<td>310</td>
<td>0.03</td>
<td>211</td>
<td>0.32</td>
<td>0.11</td>
<td>P. aeruginosa</td>
<td>[146]</td>
</tr>
<tr>
<td>Benzene</td>
<td>340</td>
<td>0.16</td>
<td>71.18</td>
<td></td>
<td></td>
<td>P. putida</td>
<td>[147]</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.22</td>
<td>0.92</td>
<td>0.48</td>
<td>0.46</td>
<td>P. putida F1</td>
<td>[145]</td>
</tr>
<tr>
<td></td>
<td>191</td>
<td>0.02</td>
<td>8.35</td>
<td>0.53</td>
<td>0.04</td>
<td>P. aeruginosa</td>
<td>[145]</td>
</tr>
<tr>
<td>Xylene (not $m$-Xylene)</td>
<td>5.00</td>
<td>0.19</td>
<td>2.55</td>
<td>0.15</td>
<td>1.27</td>
<td>P. putida F1</td>
<td>[145]</td>
</tr>
<tr>
<td></td>
<td>153</td>
<td>0.01</td>
<td>1.43</td>
<td>0.75</td>
<td>0.01</td>
<td>P. aeruginosa</td>
<td>[146]</td>
</tr>
</tbody>
</table>
In Figure 3.5, the reaction rate if given as a function of concentration. The charts indicate a high reaction rate at low concentrations for toluene, ethylbenzene and benzene (0.1 – 0.3 g m⁻³). Comparing these theoretical results to the degradation experiments in the biofilter mentioned in Chapter 3.41 and 3.42, it can be seen that the maximum achieved RE are at low inlet concentration (0.05 – 0.14 g m⁻³). Hence, a consistency between the achieved maximum RE in biofilter experiments and theoretically determined maximum reaction rates are established. For m-xylene a higher reaction rates is predicted with increasing concentration, which is supported by the conducted experiments at an EBRT of 1.5 minutes, but not at 2.5 minutes. The coefficient of determination was determined for each plot of reaction rate vs. concentration by plotting the experimental concentrations vs. the predicted concentrations. The average results are 0.94, 0.93, 0.94 and 0.88 for toluene, ethylbenzene, benzene and m-xylene, respectively.

### 3.4.3 Decay rate constant

As indicated above, no activity was measured for benzene and m-xylene biodegradation at inlet concentrations of around 0.05 g m⁻³ and 0.08 g m⁻³ respectively. Increasing the inlet concentration to 0.11 g m⁻³ and 0.14 g m⁻³ respectively, led to biofilter activity. This indicates that the threshold substrate concentration for microbial growth is on the order of 0.1 g m⁻³ for both compounds. To test this hypotheses, the decay rate constant was calculated consistent with this threshold, and the plausibility of the obtained values At the threshold concentration, the net specific growth rate is zero, which leads to the following relationship:
\[ k_d = \frac{\mu_{\text{max}} S_{\text{th}}}{K_m + S_{\text{th}} + \frac{S_{\text{th}}}{K_i}} \]  

(3.5)

where \( k_d \) represents the constant decay coefficient [h\(^{-1}\)] and \( S_{\text{th}} \) indicates the threshold substrate concentration [g m\(^{-3}\)]. The saturation constants \( K_m \) was determined in previous sections and the maximum growth rate were computed based on the product of Yield and the determined maximum reaction rate \( V_{\text{max}} \). The Yield was chosen based on literature values [134], however a wide range of values are suggested. Results were noted indicating a very high \( k_d \) value of 0.331 h\(^{-1}\) and a more plausible value of 0.00015 h\(^{-1}\) for benzene and m-xylene, respectively. It is concluded that the lack of degradation of \( m \)-xylene at low concentration is likely due to the fact that the threshold concentration for microbial growth was not reached, whereas a different mechanism is probably responsible for the lack of benzene degradation at low concentrations. An extended lag phase may have been responsible here.

### 3.5 Conclusion

In this study, various operational conditions were applied to evaluate the performance of *Nocardia* sp. and two non-hydrocarbon degrading bacteria to remove single pollutant benzene, toluene, ethylbenzene and \( m \)-xylene from waste gas streams. Experiments were conducted under two different EBRT (1.5, 2.5 min) and various average inlet concentrations (0.050 g m\(^{-3}\), 0.600 g m\(^{-3}\), 1.5 g m\(^{-3}\)). A RE of greater than 93% and 95% for an inlet loading rate (ILR) of 0.976 g m\(^{-3}\) h\(^{-1}\) and 1.697 g m\(^{-3}\) h\(^{-1}\) were reached for toluene and ethylbenzene, respectively. Also, efficiencies of 90% and higher were achieved for benzene. The maximum RE of \( m \)-xylene was 87%. These results demonstrate the
potential of *Nocardia* sp. as a suitable microbe for biodegrading single pollutant benzene, toluene, ethylbenzene and m-xylene at low concentrations. Further investigations in terms of its performance are suggested as well as the evaluation of its capability to treat a mixture of BTEX in a waste gas stream.
4 Biological treatment of waste gases contaminated with a BTEX mixture and determination of kinetic parameters

4.1 Abstract

The biological treatment of an air stream contaminated with a VOC blend of BTEX by a consortium of one BTEX degrading and two non BTEX degrading bacteria was investigated. The proportions of each VOC in the inlet air stream were 35%, 36%, 5% and 24% for benzene, toluene, ethylbenzene and m-xylene, respectively, representative of a typical BTEX containing waste gas. The compounds ranked as follows in decreasing order of biodegradability at an EBRT of 1.5 min: toluene, ethylbenzene, benzene and m-xylene, with maximum removal efficiencies (RE) of 91.5%, 90.5%, 84% and 30.5%, respectively. With an increase of EBRT to 2.5 min the order changed and ethylbenzene was removed with a higher efficiency than toluene: 95% to 94%, which corresponds to elimination capacities of 1.46 g m$^{-3}$ h and 5.29 g m$^{-3}$ h at low concentration of VOC mixture. In addition, kinetic parameters were determined and revealed some inconsistency with literature values. However, the biofilter system is comparable or exceeding others. Hence, the biofilter here tested is able to treat a polluted air stream containing BTEX.
4.2 Introduction

Pollutants emitted to the environment can pose a great adverse effect to human health and the ecosystem. Air contaminants such as volatile organic compounds (VOCs), among others, originate anthropogenically from different industrial sources or naturally. VOCs are often defined as having a vapor pressure of 0.01 kPa or more at 293.15 K (20°C). Another way to define VOCs, is in terms of the molecules’ boiling points, which would be under 250°C at 101 325 Pa. Benzene, toluene, ethylbenzene and xylene represent molecules of VOCs and are collectively called BTEX. These compounds are toxic, carcinogenic and mutagenic molecules [148] and also listed as Hazardous Air Pollutants (HAPs) in the US Clean Air Act Amendments [149] and are considered toxic under the Canadian Environmental Protection Act, 1999 [150].

Available physical or chemical technologies used to treat VOCs, and hence BTEX, are oxidation, adsorption, absorption, membrane separation, condensation and non-thermal plasma, which effectively treat the targeted emissions from various industrial sources [151–156]. But the downside of these techniques is the potential generation of unwanted byproducts. In addition, operational costs and the energy demand can be higher for treating high gas flow rates (>1000 m³ h⁻¹) and low concentration (< 1 g m⁻³) gas streams [68,157–159] compared to a biofilter. Specific bacteria and fungi have been reported to degrade BTEX as well as consortia of microorganisms. In both cases, removal efficiencies >90%, often >95%, were achieved [110,112,160,161]. If a biofilter is inoculated with an unknown consortium of microbes, the anticipated removal efficiency might not be high enough to comply with regulation, although microbes capable of degrading the prevailing contaminants will proliferate and a natural hierarchy will occur. In turn, to maintain a single
bacterium in a biofilter, possible contaminates need to be removed from the waste gas prior to entering the biofilter, otherwise, potential interaction of microbes lead to a change in efficiency. However, such a change could increase the efficiency or decrease it, as shown in other biological systems such as bacteria-algae systems [162]. If the interaction has a negative impact the anticipated removal efficiency might not comply with regulations.

When a single bacterium is used, the possible negative impact needs to be avoided. Therefore, a BTEX degrading bacterium, *Nocardia* sp., was tested under the presence of two non-BTEX degraders on its capabilities to degrade BTEX and conduces as a first step to improve the biofilter process.

### 4.3 Material and methods

#### 4.3.1 Microorganism and filter media

A sterilized mixture of compost and straw (vol% 80/20) was used as filter bed material. Compost and straw are natural products. In Table 4.1, the filter bed properties are shown and analysis was conducted by AGAT Laboratories. All used chemicals; benzene, toluene, ethylbenzene, m-xylene, and solids for the cultivation medium were purchased from Sigma Aldrich. The sterilized adapted liquid BH-Medium [123] contained 1 g L⁻¹ KH₂PO₄, 1 g L⁻¹ Na₂HPO₄, 0.5 g L⁻¹ NH₄NO₃, 0.002 g L⁻¹ FeCl₃, 0.002 g L⁻¹ MnSO₄ 2H₂O, 0.2 g L⁻¹ MgSO₄ 7H₂O and 0.02 g L⁻¹ CaCl₂ 2H₂O. As a carbon source, 1μL of each compound of BTEX were used.

In order to introduce microbes into the inoculant containing *Nocardia* sp., an air stream was piped into the serum bottles. After three days, plating of cells were conducted and
standard procedure were followed [135]. Results indicating one microbe was able to
degradate toluene as only available carbon source, which is *Nocardia* sp., since this the
indigenous bacterium in the pure inoculant. The other two bacteria were tested on their
ability to biodegrade toluene as only available carbon source and were not able to do so.
Therefore, it is assumed that *Nocardia* sp. is the only BTEX degrading microbe in the
mixture. No further characterization of microbes was conducted. *Nocardia* sp. as BTEX
degrading and two non-BTEX degrading microbes were cultivated under ambient
temperature (21-22°C) in serum bottles sealed with a butyl rubber septum and aluminum
crimp seal and containing a sterilized liquid cultivation medium. Experimental trials were
conducted under an ambient temperature between 21-22°C.

<table>
<thead>
<tr>
<th>Filterbed properties</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Nitrogen</strong></td>
<td>15900</td>
<td>mg kg⁻¹</td>
</tr>
<tr>
<td><strong>NO₃ - N and NO₂-N</strong></td>
<td>916</td>
<td>mg kg⁻¹</td>
</tr>
<tr>
<td><strong>Organic Matter</strong></td>
<td>29.50</td>
<td>%</td>
</tr>
<tr>
<td><strong>PO₄-P</strong></td>
<td>194</td>
<td>mg kg⁻¹</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>7.30</td>
<td>-</td>
</tr>
<tr>
<td><strong>Particle Size Distribution</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2000-50 μm)</td>
<td>44</td>
<td>%</td>
</tr>
<tr>
<td>(50-2 μm)</td>
<td>37</td>
<td>%</td>
</tr>
<tr>
<td>(&lt;2 μm)</td>
<td>19</td>
<td>%</td>
</tr>
</tbody>
</table>

### 4.3.2 Biofilter set-up and experimental conditions

The BTEX biodegradation was carried out in a lab-scale biofilter with an inoculation
volume of 100 mL and an OD₆₅₀ of around 0.2. Biofilter columns were made of
polycarbonate with a total height of 63 cm and an internal diameter of 10 cm. Glass beads
at a total height of 5 cm and a 1 cm thick perforated polycarbonate plate were used to evenly distribute the inlet gas stream at the inlet. Sampling ports to measure gas samples were centered at the top and bottom of the column and sealed with GC septa (0.95 cm diameter). The filter bed material was filled up to a total height of 25 cm and placed in a fume hood. In Figure 4.1, a schematic of the setup is shown. To generate one air stream, an air pump (Pond Master Ap-40) was used, which was split and sent through two gas washing bottles. One was filled with sterilized tap water and the other filled with the contaminant. The two gas flows were combined and mixed in an empty gas-washing bottle before being introduced into the biofilter. The total flow was controlled by two rotameters (Cole-Parmer) located after the gas flow split and the total flow rate was measured (TI-400) prior the sample port and inlet. The operational parameters for the biofilter are summarized in Table 4.2. An adsorption test for single BTEX compounds were conducted prior to the experiments presented here and no adsorption was observed based on the sterilized filterbed material or Plexiglas. In Appendix A, the adsorption test results are shown.
Figure 4.1: Schematic of experimental set-up: 1) air pump, 2) rotameters, 3) water vessel, 4) benzene vessel, 5) toluene vessel, 6) ethylbenzene vessel, 7) m-xylene vessel, 8) mixing vessel, 9) flowmeter and 10) biofilter
Table 4.2: Operational parameter for EBRT of 1.5 min and 2.5 min and VOC range of inlet concentration

<table>
<thead>
<tr>
<th>EBRT [s]</th>
<th>range of BTEX inlet concentration [g m⁻³]</th>
<th>benzene</th>
<th>toluene</th>
<th>ethylbenzene</th>
<th>m-xylene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>low conc.</td>
<td>0.247 - 0.348</td>
<td>0.207 - 0.320</td>
<td>0.016 - 0.062</td>
<td>0.142 - 0.235</td>
</tr>
<tr>
<td></td>
<td>high conc.</td>
<td>0.498 - 0.671</td>
<td>0.432 - 0.470</td>
<td>0.081 - 0.096</td>
<td>0.260 - 0.348</td>
</tr>
<tr>
<td>90</td>
<td>low conc.</td>
<td>0.210 - 0.352</td>
<td>0.153 - 0.271</td>
<td>0.033 - 0.073</td>
<td>0.121 - 0.218</td>
</tr>
<tr>
<td></td>
<td>high conc.</td>
<td>0.438 - 0.540</td>
<td>0.541 - 0.603</td>
<td>0.039 - 0.130</td>
<td>0.193 - 0.323</td>
</tr>
<tr>
<td>150</td>
<td>low conc.</td>
<td>0.157 - 0.279</td>
<td>0.185 - 0.248</td>
<td>0.016 - 0.040</td>
<td>0.129 - 0.292</td>
</tr>
<tr>
<td></td>
<td>high conc.</td>
<td>0.501 - 0.593</td>
<td>0.458 - 0.631</td>
<td>0.039 - 0.099</td>
<td>0.235 - 0.307</td>
</tr>
</tbody>
</table>

An initial total VOC concentration of 0.800 g m⁻³ was maintained and increased to 1.5 g m⁻³ in the second step. The proportions of VOCs in the blend are 35%, 36%, 5% and 24% for benzene, toluene, ethylbenzene and m-xylene, representative of an industrial BTEX containing waste gas.

4.3.3 Kinetic batch tests

The kinetic characterization of the inoculated bacteria was performed under sterile conditions in batch tests. The batch tests were conducted in 160 mL serum bottles sealed with a butyl rubber septum and aluminum crimp seal. Each serum bottle were filled with 100 mL of sterilized adapted liquid BH-medium and suspended cells (OD₆₅₀ about 0.1), which were previously adapted to a BTEX mixture. The initial liquid volume of benzene, toluene, ethylbenzene and m-xylene added to the serum bottles was 1 µL for each compound. The biodegradation tests were carried out under room temperature (21-22°C) and the medium were continuously mixed to avoid mass transfer limitation. Duplicates of each test were performed and the average number of the results are presented further down.
Adsorption due to the liquid phase were not observed as control test were carried out. Results are shown in Appendix B. The gas phase concentration was measured, however kinetic parameters are represented for the liquid phase. Measurements were taken after injecting the liquid contaminate and the absence of a lag-phase was seen for some trials. Hence, for analyzing the experimental data, a lag phase simulation was conducted following the approach outlined in [136]. Assuming that Henry’s law applies, the differential equation to be integrated numerically is:

\[ \left( \frac{V_L}{H} + V_G \right) \frac{dC_G}{dt} = -m \cdot r \]  \hspace{1cm} (3.1)

where \( V_L \) is the volume of the liquid phase \([m^3]\), \( V_G \) is the volume of the gas phase \([m^3]\), \( H \) is the Henry constant \([\ ]\), \( C_G \) is the gas phase concentration of toluene \([g \cdot m^{-3}]\), \( t \) is the time \([s]\), \( m \) the biomass \([g]\), and \( r \) is the BTEX degradation rate.\([g \cdot m^{-3} \cdot h^{-1}]\). Hence, \( r \) in equation was replaced by \( \alpha \), where \( \alpha \) is given by:

\[ \alpha = \frac{q_0}{q_0 + e^{(-\nu t)}} \]  \hspace{1cm} (3.2)

The reaction rate \( r \) is given by:

\[ r = \frac{V_{\text{max}} C_L}{K_m + C_L + \frac{C_L^2}{K_I}} \]  \hspace{1cm} (3.3)

where \( C_L = C_G/H \).

The numerical integration was carried out by Euler’s method in Excel, and the parameters \( V_{\text{max}} \), \( K_m \), and \( K_I \) as well as \( q_0 \) and \( \nu \) were determined by nonlinear least squares, using the solver function in Excel.
4.3.4 Analytical methods

To take and inject a 200 µL gas sample, a SGE 250 µL gastight syringe was used. The gas samples were analyzed with a gas chromatograph (GC-2014, Shimadzu) equipped with an FID and Rtx®-Wax capillary column (30 m x 0.53 mm x 1 µm). The injector and detector temperatures were set at 250°C. The oven Temperature was set to 75°C isothermal.

4.3.5 Performance parameter

The performance of a biofilter is commonly expressed by the elimination capacity (EC), the removal efficiency (RE), inlet loading rate (ILR) and empty-bed residence time (EBRT) and those parameters are defined as follows:

\[
EC = \frac{Q (C_i - C_o)}{V} \quad (2.7)
\]

\[
RE = \frac{C_i - C_o}{C_i} \times 100 \quad (2.6)
\]

\[
ILR = \frac{Q C_i}{V} \quad (2.5)
\]

\[
EBRT = \frac{V}{Q} \quad (2.1)
\]

were \(C_i\), \(C_0\), \(Q\), and \(V\) representing the inlet concentration [g m\(^3\)], outlet concentration [g m\(^3\)], flow rate [g m\(^3\)] and volume [m\(^3\)], respectively.
4.4 Results and discussion

4.4.1 Biofilter experiment

4.4.1.1 Overall BTEX removal

In order to ensure the observed trends were not random, the first phase of the experiment, with an EBRT of 1.5 min, was repeated with new autoclaved filter bed material and inoculant. In Figure 4.2 the first and the duplicate run at an EBRT of 1.5 min, low and high total BTEX inlet concentration are shown. Only RE is shown to illustrate the similarity between the trials. RE, inlet and outlet concentrations of both runs are shown in Figure 4.3 and only the duplicate run is depicted in Figure 4.4. It can be seen that the REs of the different trials are similar and, therefore the measured activity in terms of inlet and outlet concentration are not randomly. Hence, at an EBRT of 2.5 min the trial was only conducted once. Furthermore, during the first run, steady RE were reached faster, due to a higher number of inoculated microorganisms.
Figure 4.2: Consistency of biofil-te set-up verified by two independent runs under EBRT of 1.5 min, a) low inlet concentration of total BTEX and b) high inlet concentration of total BTEX
When comparing the first run and the duplicate at an EBRT of 1.5 min and low total VOC concentration, the achieved maximum REs are 90% and 93% for toluene, 91% and 90% for ethylbenzene, 82% and 86% for benzene and 30% and 31% for m-xylene. This corresponds to an average maximum RE of 91.5%, 90.5%, 84% and 30.5% for toluene, ethylbenzene, benzene and m-xylene, respectively, and corresponds to an average maximum EC of 8.5 g m⁻³ h⁻¹, 1.44 g m⁻³ h⁻¹, 11.19 g m⁻³ h⁻¹ and 2.21 g m⁻³ h⁻¹, which lead to an EC sum of 23.34 g m⁻³ h⁻¹. Therefore, toluene and ethylbenzene are the most favorable degraded compounds. An increase of concentration led to a decrease of average maximum RE of 88.5%, 83.5%, 67% and 25% for ethylbenzene, toluene, benzene and m-xylene, respectively. The ECs are 2.42 g m⁻³ h⁻¹, 18.41 g m⁻³ h⁻¹, 13.94 g m⁻³ h⁻¹ and 2.56 g m⁻³ h⁻¹, respectively. The degree of degradation indicates the persistence of benzene for biodegradation compared to ethylbenzene and toluene. Similar results were found in [77].

The illustrated recalcitrant effect of m-xylene was observed in other studies at similar EBRT [161]. In addition, the rise of concentration caused a higher decline in RE for toluene and hence ethylbenzene is more favorable than toluene in terms of degradation under a higher EBRT. A general decline in REs with increasing ILR were expected and is consistent with other findings [110,161].
Figure 4.3: RE, inlet and outlet concentration under different EBRT for single compounds, benzene in a, toluene in b, ethylbenzene in c and m-xylene in d.
Figure 4.4: Duplicate run at 1.5 min EBRT for low total VOC concentration; benzene in a, toluene in b, ethylbenzene in c and m-xylene in d.
With an increase of the EBRT to 2.5 min, the observed maximum RE generally increased, which was expected. The highest maximum achieved REs at low VOC inlet concentration and an EBRT of 1.5 min are 95%, 94%, 81% and 36% for ethylbenzene, toluene, benzene and m-xylene, respectively. This corresponds to a maximum EC of 1.46 g m⁻³ h⁻¹, 5.29 g m⁻³ h⁻¹, 6.71 g m⁻³ h⁻¹ and 1.08 g m⁻³ h⁻¹, respectively. The order of biodegradation as the EBRT was increased were approximately the same, since the different in achieved REs for ethylbenzene and toluene was only 1%.

The efficiency of benzene biodegradation dropped by 3% as EBRT was increased, which could be explained by an antagonistic substrate effect. Since total VOC and single BTEX concentration increased, an inhibiting effect might have occurred. Conducted experiments identify the type of interaction between BTEX pollutants and, for example, an inhibiting effect was indicated between toluene and p-xylene [163]. A different study concluded the decline in degradation performance of toluene in the presence of benzene, ethylbenzene or xylenes [164].

4.4.1.2 Comparison to single BTEX experiments

The obtained results are compared to the findings indicated in Chapter 3, where single BTEX compounds were tested with the same biofilter setup and inoculant. When benzene is treated in a mixture it is the second least favorable compound (81% and 84% - EC 11.19 and 6.7 g m⁻³ h⁻¹) for biodegradation under both tested EBRTs (1.5 min and 2.5 min) and inlet concentrations between 0.200 and 0.500 g m⁻³. Tested as single compound at an EBRT of 1.5 min, the maximum achieved RE was 100% (max EC of 5.07 g m⁻³ h⁻¹), however at an inlet concentration at around 0.110 g m⁻³. Due to the lower inlet concentration compared
to the benzene treatment in a mixture, the EC is lower even though the maximum achieved
RE is higher. In addition, under an EBRT of 2.5 min the maximum RE was 95% (max EC
3.56 g m\(^{-3}\) h\(^{-1}\)). This potentially indicates an antagonistic effect of toluene, ethylbenzene or
m-xylene on the biodegradation rate of benzene. Hence, aforementioned antagonistic
behavior of TEX on benzene is potentially supported with this result, considering the
different inlet concentrations.

Toluene, as a single contaminant, under each tested set of parameters except for low inlet
concentration and low EBRT, was the most favorable molecule for biodegradation based
on the obtained RE, which corresponds with the results of the treated BTEX mixture. For
the second-most favorable compound, ethylbenzene, a similar result can be observed. The
most persistent molecule in terms of biodegradability was m-xylene in all trials. Hence,
hypothetically there is no significant synergistic effect on m-xylene based on the presence
of BTE. Based on the determined low \( K_m \) and large \( K_I \) values in a BTEX mixture, in
Chapter 4.2, a low affinity and no indication of substrate inhibition is observed. Therefore,
it is assumed that the molecular structure of \( m \)-xylene prevents easy access for the enzyme,
which likely prefers to bind with a more easily accessible substrate, in this case toluene,
benzene or ethylbenzene.

### 4.4.2 Kinetics and parameter estimation

In order to determine the kinetic parameter of Haldane-kinetics, equation 4.7 (essentially
equation 2.47), was applied to study single substrate biodegradation.

\[
-r' = \frac{V_{\text{max}} c}{K_m + c + \frac{c^2}{K_I}}
\]  

(3.4)
Where $r$ represents the reaction rate $[\text{g m}^{-3} \text{ h}^{-1}]$, $c$ is the concentration of contaminant $[\text{g m}^{-3}]$, $V_{\text{max}}$ is the maximum reaction rate $[\text{g m}^{-3} \text{ h}^{-1}]$, $K_m$ expresses the half-saturation constant $[\text{g m}^{-3}]$ and $K_I$ is the inhibition constant $[\text{g m}^{-3}]$. In order to simulate the lag phase, the method of Jozef Baranyi [136] was applied.

The computed average parameters are represented in Table 4.3 and corresponding figures are depicted in Figure 3.5. In addition, for each batch test the computed reaction rate as a function of time is shown in Figure 4.6. Here it is necessary to emphasize that the literature values for xylene are not specifically those for m-xylene, since none were found.
### Table 4.3: Computed parameter of this study

<table>
<thead>
<tr>
<th></th>
<th>$K_I$</th>
<th>$V_{\text{max}}$</th>
<th>$K_m$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[g m$^{-3}$]</td>
<td>[g g$^{-1}$ h$^{-1}$]</td>
<td>[g m$^{-3}$]</td>
<td></td>
</tr>
<tr>
<td>Toluene</td>
<td>253</td>
<td>0.33</td>
<td>0.03</td>
<td>0.96</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>5.25</td>
<td>0.88</td>
<td>0.06</td>
<td>0.95</td>
</tr>
<tr>
<td>Benzene</td>
<td>12</td>
<td>0.30</td>
<td>0.02</td>
<td>0.94</td>
</tr>
<tr>
<td>$m$-Xylene</td>
<td>57</td>
<td>0.11</td>
<td>0.01</td>
<td>0.94</td>
</tr>
</tbody>
</table>

### Table 4.4: Literature values of kinetic parameters

<table>
<thead>
<tr>
<th></th>
<th>$K_I$</th>
<th>$\mu_{\text{max}}$</th>
<th>$K_m$</th>
<th>Yield</th>
<th>$V_{\text{max}}$</th>
<th>Microbe</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[g m$^{-3}$]</td>
<td>[h$^{-1}$]</td>
<td>[g m$^{-3}$]</td>
<td>[g bio g substrate$^{-1}$]</td>
<td>[g g$^{-1}$ h$^{-1}$]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toluene</td>
<td>42</td>
<td>0.42</td>
<td>3.98</td>
<td>0.93</td>
<td>0.45</td>
<td>P. Putida 54G consortia</td>
<td>[143]</td>
</tr>
<tr>
<td></td>
<td>78</td>
<td>0.86</td>
<td>11.03</td>
<td>0.71</td>
<td>1.21</td>
<td>P. putida</td>
<td>[144]</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>0.72</td>
<td>15.07</td>
<td>0.64</td>
<td>1.13</td>
<td>P. putida F1</td>
<td>[144]</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>0.46</td>
<td>0.65</td>
<td>0.95</td>
<td>0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>20</td>
<td>0.26</td>
<td>1.50</td>
<td>0.40</td>
<td>0.65</td>
<td>P. putida F1</td>
<td>[145]</td>
</tr>
<tr>
<td></td>
<td>310</td>
<td>0.03</td>
<td>211</td>
<td>0.32</td>
<td>0.11</td>
<td>P. aeruginosa</td>
<td>[146]</td>
</tr>
<tr>
<td>Benzene</td>
<td>340</td>
<td>0.16</td>
<td>71</td>
<td>0.48</td>
<td>0.46</td>
<td>P. putida</td>
<td>[147]</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.22</td>
<td>0.92</td>
<td>0.46</td>
<td>0.46</td>
<td>P. putida F1</td>
<td>[145]</td>
</tr>
<tr>
<td></td>
<td>191</td>
<td>0.02</td>
<td>8.35</td>
<td>0.53</td>
<td>0.04</td>
<td>P. aeruginosa</td>
<td>[146]</td>
</tr>
<tr>
<td>Xylene not $m$-xylene</td>
<td>5.00</td>
<td>0.19</td>
<td>2.55</td>
<td>0.15</td>
<td>1.27</td>
<td>P. putida F1</td>
<td>[145]</td>
</tr>
<tr>
<td></td>
<td>153</td>
<td>0.01</td>
<td>1.43</td>
<td>0.75</td>
<td>0.01</td>
<td>P. aeruginosa</td>
<td>[146]</td>
</tr>
</tbody>
</table>

Comparison to literature values, shown in Table 4.4 revealed a difference, since computed parameters are not in the range of those found in literature. However, the literature values were obtained by using only a single substrate. For instance all $K_m$ values are significantly smaller as those found in the literature, which indicates a higher affinity to the tested compound. $K_I$ values are $3$ times larger for toluene and smaller for ethylbenzene and benzene. However, the result for ethylbenzene is $4$ times smaller, whereas the benzene result is one order of magnitude smaller. Hence, the substrate inhibition effect at high
concentration is more pronounced for ethylbenzene than for benzene, according to obtained results. In addition, it is indicated that toluene inhibition is higher compared to the literature results. Despite the differences for $K_m$ and $K_I$, values of $V_{max}$ are coherent with those indicated from other studies. In addition, the half saturation constant $K_m$ and inhibition constant $K_I$ for m-xylene are consistent with the literature findings, considering the fact $m$-xylene is compared to xylene. The revealed differences between prevailing study and literature values, might be based on the different microbes used.
Figure 4.5: Batch test for determination of kinetic parameters and prediction, a) toluene, b) ethylbenzene, c) benzene and d) m-xylene
Reaction rate $[\text{g m}^{-3} \text{h}^{-1}]$

Concentration $[\text{g m}^{-3}]$

ai)

Reaction rate $[\text{g m}^{-3} \text{h}^{-1}]$

Concentration $[\text{g m}^{-3}]$

aii)
bi) Reaction rate \([\text{g m}^{-3} \text{h}^{-1}]\) vs Concentration \([\text{g m}^{-3}]\)

bii) Reaction rate \([\text{g m}^{-3} \text{h}^{-1}]\) vs Concentration \([\text{g m}^{-3}]\)
(a) Reaction rate $[\text{g m}^{-3} \text{h}^{-1}]$

$$\begin{array}{c}
\text{Concentration [g m}^{-3}] \\
\hline
0 & 0.5 & 1 & 1.5 & 2 & 2.5 & 3 \\
\hline
0 & 0.5 & 1 & 1.5 & 2 & 2.5 & 3 \\
\hline
\end{array}$$

(b) Reaction rate $[\text{g m}^{-3} \text{h}^{-1}]$

$$\begin{array}{c}
\text{Concentration [g m}^{-3}] \\
\hline
0 & 0.5 & 1 & 1.5 & 2 & 2.5 & 3 \\
\hline
0 & 0.5 & 1 & 1.5 & 2 & 2.5 & 3 \\
\hline
\end{array}$$
Comparing the obtained results to those in Chapter 3 it is obvious that for all four compounds $V_{\text{max}}$ did decrease. The decline was 53%, 46%, 40% and 8% for benzene, toluene, m-xylene and ethylbenzene, respectively. A possible explanation could be the
impact of different substrates at the same time. $K_I$ and $K_m$ did change as well for each compound. However, the smallest change of $K_I$, $K_m$ and $V_{\text{max}}$ was observed for ethylbenzene. Therefore, the effect of different substrates on kinetic parameters and consequently on biodegradation behavior was very little compared to the effect on other compounds. This is supported by considering the time needed to degrade the injected ethylbenzene as a single compound and in a mixture. In both cases, biodegradation of ethylbenzene was the fastest. Generally, a decline of $K_m$ is observed in this study, compared to single BTEX batch tests. Hence, the affinity to the respective compound did increase. In addition, the substrate inhibition effect at high concentration decreased for toluene and benzene, while for m-xylene it increased. Similar results were acquired for ethylbenzene. However, because the $K_I$ value is still far outside the range of experimental concentrations, the decrease of $K_I$ for m-xylene is probably not meaningful.

Considering the plot of reaction rate as a function of concentration, it can be seen that some of them showed a trend similar to Haldane-kinetic and others not. This deviation might be due to the impact of the other compounds on kinetics. The coefficient of determination was determined for each plot of reaction rate vs. concentration by plotting the experimental concentrations over the predicted concentrations. The average results are 0.96, 0.95, 0.94 and 0.94 for toluene, ethylbenzene, benzene and m-xylene, respectively. It also can be seen, that the highest reaction rates are obtained at low concentration. This finding is consistent with conducted biofilter experiments treating a mixture of BTEX.
4.5 Conclusion

The presented results of an engineered microbial system, containing one BTEX degrader, *Nocardia sp.*, and two non BTEX degraders show an effective method to treat a gas stream blended with BETX. At lower inlet concentrations and low EBRT (1.5 min) the average maximum RE for toluene and ethylbenzene was > 90%, with an average maximum EC of 8.5 g m\(^{-3}\) h and 1.44 g m\(^{-3}\) h\(^{-1}\), respectively. For benzene and m-xylene, average maximum REs of 84% and 30.5%, respectively, were achieved. With increasing EBRT, the RE rose. The same behavior was observed at higher EBRT and higher inlet concentrations, whereas, generally, the RE did increase as well. In addition, the acquired kinetic parameter indicated some differences compared to literature values. However, by comparing the biofilter to other systems, it is capable of treating a gaseous blend of BTEX.
5 Multiple steady states in a diffusion-limited biofilm of a VOC treating biofilter

5.1 Abstract

The occurrence of multiple steady states in a toluene biodegrading, diffusion-limited biofilm under aerobic conditions was investigated by computer simulation. Two independent simulation models were used: one steady-state, and one nonsteady-state. The models identified two stable steady states and one unstable intermediate steady state in a limited parameter space. The nonsteady-state model predicts conditions that evolve to a steady state that is within 1% of the solution of the steady-state model, confirming the used algorithms are valid. Multiple steady states occur if, 1) a single biofilm is exposed to a constant external pollution concentration, which exceeds or undershoots a certain threshold, 2) under a limiting parameter space and 3) provided that the pollutant degradation follows Haldane kinetics (substrate inhibition). With both models, apparent reaction rates, effectiveness factors and concentration profiles in the biofilm can be computed. In addition, a biofilm in a state of bifurcation, under aforementioned conditions, displays Michaelis-Menten-like falsified kinetics from a concentration range starting at zero up to the occurrence of a second steady state. For both findings, the emergence of multiple steady states and falsified kinetics can negatively affect the operation of a biofilter and experimental determination of kinetic parameters, respectively.
5.2 Introduction

With growing awareness of hazards related to gaseous emissions released into the environment and their magnitude, the demand for pollution control technologies and regulations is increasing. One of the concerning compounds released by industries such as oil and gas, chemicals, pulp and paper, etc. are Volatile Organic Compounds (VOCs). Benzene, toluene, ethylbenzene and xylenes (BTEX) are among these VOCs and have major adverse effects. Benzene, for example, is considered to be carcinogenic [13]. Physical and chemical technologies are available to reduce the burden caused by VOCs, but are often expensive or can generate undesirable by-products. In contrast, biofiltration has low operational and equipment costs and does not produce unwanted by-products.

In a biofilter, microorganisms adhere to a synthetic, inorganic or organic surface of the filter media and act as a biocatalyst. Metabolic activity of prevailing microorganisms has a great impact on the VOC removal efficiency, as do factors such as inlet concentration, moisture content, pH etc. on the metabolic activity. The treatment of a single compound VOC [53,74,81,137,165,166] and mixtures [57,110,167,168] have been evaluated in lab-scale or pilot-scale.

In addition, computer simulation models have been developed to aid biofilter design and predict the behavior of a system. Ottengraf and Van Den Oever [117] were one of the first who developed a biofilter model. Assumptions made in their model were e.g. 1) the resistance at the interface between the biolayer and the gas phase is negligible hence, Henry’s law is applicable, 2) nutrient transport in the biolayer occurs by diffusion, 3) compared to the diameter of packing the particle, the thickness of the biofilm is negligible, 4) substrate degradation kinetics follows zero-order kinetics, and 5) the gas flow can be
considered as plug flow. This basic biofilter model does not account for substrate inhibition effects or biomass growth. Shareefdeen et al. [118] developed the first steady-state biofilter model to describe methanol vapor degradation and one assumption was that oxygen and substrate were reaction rate limiting components. The dynamic model developed by Deshusses and Dunn [119,120] includes pollutant interaction and considered biodegradation of methyl ethyl ketone and methyl isobutyl ketone in a downward flow biofilter. One of the advantages is that it incorporates substrate inhibition and substrate interaction and it accounts for diffusion limitation. Li and De Visscher [122] developed a toluene biofilter model that combines Haldane kinetics with the Verhulst equation for microbial growth. The main advantage is that it describes changes in activity as a result of changing concentration or flow rate. De Visscher et al. [169] replaced the Verhulst portion of the model of Li and De Visscher [122] with a nitrogen dynamics and limitation model. This model includes toluene limitation but not oxygen limitation. Because of the assumption of a variable biofilm thickness, the model has the flexibility of deciding whether or not diffusion limitation should be considered on the calculated toluene degradation.

As shown in the literature, the development of simulation models that describe the behavior and performance of an actual biofilter has been given much effort [31,147,170,171]. In other industrially utilized biological processes such as biofilm reactors, much effort has been spent on process simulation as well [172,173]. For example, in a biofilm reactor, multiple steady states were mathematically investigated [174,175].

In Russo et al., [174] a three-phase internal loop airlift biofilm reactor is represented in terms of conversion of phenol by immobilized cells of *Pseudomonas* sp. OX1. The model
incorporates adhesion of cells, growth of attached cells, and biofilm detachment. Adhesion of suspended cells is described by first-order kinetics and the linear relationship between biofilm detachment rate and biofilm loading was assumed in order to describe the effect of abrasion due to particle collisions. Multiple steady states were confirmed based on cooperative and competitive effects between immobilized and free cells. This proposed model was further developed by Olivieri et al. [175] and comprises double-substrate kinetics for cell and biofilm growth, description of substrate conversion in the biofilm as a parameter for the reactor performance and, as in the previous model described, cell adhesion and biofilm detachment. Two different conversion regimes were identified, one controlled by the free cells and one controlled by the immobilized cells. For both regimes, multiple steady states were indicated. The regime controlled by immobilized cells was more complex in terms of multiplicity and stability of individual steady states.

The reason for multiple steady states could be explained by different considerations, for example, mixing of the contaminant through the liquid media, diffusion into the biofilm and the effects of free cells and immobilized cells. However, the possibility of multiple steady states when only substrate degradation in a diffusion-limited biofilm is considered has not been investigated to date. Multiple steady states are known to occur in some chemostat-type processes [176].

Based on simulation results, it is known that the concentration of signaling molecules in quorum sensing displays two steady states. This occurs through an autocatalytic process regulated by the diffusion of the signaling molecules through the cell wall. The two steady states are hypothesized to be responsible for the switching mechanism between two metabolic states (e.g., virulent or non-virulent) [177]. The term kinetic bifurcation is
sometimes used to indicate multiple steady states. However, because the term bifurcation more commonly refers to oscillating or chaotic reaction dynamics, a phenomenon unrelated to the multiple steady states studied here, the term will not be used in this work.

This Chapter establishes that a single biofilm exposed to a constant external pollutant concentration can have two steady states in a limited parameter space, provided that the pollutant degradation kinetics follows Haldane kinetics (substrate inhibition). Consequently, a biofilm can potentially undergo a sudden alteration in removal efficiency as it jumps from one steady state to the other. The steady states have 1) two different diffusion profiles and hence different concentrations at the inside of the biofilm, 2) two different overall reaction rates, and hence, 3) two different effectiveness factors. In addition, it is demonstrated that a biofilm with Haldane kinetics capable of two steady states displays Michaelis-Menten-like falsified kinetics from zero concentration up to the concentration where the high-activity steady state is no longer stable, and drops to a low-activity steady state.

5.3 Model description

The occurrence of two or more steady states has been studied in biofilm reactors, as aforementioned, and has been well established. However, the presence of more than one steady state in a biofilter biofilm has not been studied yet. This case is solely based on the diffusion of contaminant in the biofilm, degrading a pollutant with Haldane kinetics. A steady-state and a nonsteady-state model were developed to determine the steady states. The primary function of the steady-state model is to indicate the occurrence of multiple
steady states, based on the concentration at the surface of the biofilm and consequently the concentration in the gas phase. The nonsteady-state model determines which steady state will be reached given a prior history of the concentration in the system. Both models can calculate the overall reaction rates, effectiveness factors and concentration profiles in the biofilm. The two models are independent. Hence, a comparison of the outputs of both models can be used to validate the numerical aspects of the algorithms used. The following assumptions were made:

1. Since the gas phase concentration is in a low range, the gas-biofilm equilibrium is described by Henry’s law.
2. A planar (slab) geometry of the biofilm is assumed.
3. Substrate biodegradation and substrate inhibition was assumed to follow Haldane kinetics.
4. Two Models: a non-steady state and steady state
5. Oxygen is available in excess and not considered as a limiting factor.

### 5.4 Model development

For the steady-state and nonsteady-state models molecular diffusion in the biofilm is governed by Fick’s law:

\[
J = -D_A \frac{dc_A}{dx}
\]  

(5.1)

were \(J\) is the flux of component A. \(D_A\) represents the diffusion coefficient of component A [\(\text{g}_{\text{substrate}} \text{ m}^{-2} \text{ h}^{-1}\)], \(c_A\) refers to the concentration of the compound A [\(\text{g}_{\text{substrate}} \text{ m}^{-3}\)] in the biofilm, and \(x\) represents the length coordinate [m] in the direction of the biofilm thickness.
Also, for both models, the biodegradation rate of toluene was assumed to follow Haldane kinetics, which considers substrate inhibition. It calculates the reaction rate as follows:

\[ r = -\frac{V_{\text{max}} \cdot c_A \cdot \rho_{\text{bio}}}{K_s + c_A + \frac{c_A^2}{K_i}} \]  

(5.2)

\( V_{\text{max}} \), \( K_s \), \( K_i \) and \( \rho_{\text{bio}} \) express the maximum toluene degradation rate per unit weight of biomass per hour [g\(_{\text{substrate}}\) g\(_{\text{dw}}\) substrate degrading biomass\(^{-1}\) h\(^{-1}\)], the Michaelis-Menten constant [g\(_{\text{substrate}}\) m\(^{-3}\)], the inhibition constant [g\(_{\text{substrate}}\) m\(^{-3}\)] and the biomass density in the biofilm [g\(_{\text{biomass}}\) m\(^{-3}\)], respectively.

For the non-steady state model, a mass balance of the system, considering the biofilm, leads to the following equation:

\[ \frac{\partial c_A}{\partial t} = D_A \frac{\partial^2 c_A}{\partial x^2} - \frac{V_{\text{max}} \cdot c_A}{K_s + c_A + \frac{c_A^2}{K_i}} \cdot \rho_{\text{bio}} \]  

(5.3)

Next, define:

\[ x = x' \cdot L \]  

(5.4)

where \( L \) is the biofilm thickness [m], \( x \) represents the distance coordinate in the biofilm [m], and \( x' \) is a dimensionless distance coordinate in the biofilm. The substitution of equation (5.4) into equation (5.3) lead to the following term:

\[ \frac{\partial c_A}{\partial t} = \frac{D_A}{L^2} \frac{\partial^2 c_A}{\partial x'^2} - \frac{V_{\text{max}} \cdot c_A}{K_s + c_A + \frac{c_A^2}{K_i}} \cdot \rho_{\text{bio}} \]  

(5.5)

The concentration profile in the biofilm can be calculated with equation (5.5) by solving the partial differential equation. The boundary conditions are as follows:

\[ c_A |_{x=1} = \frac{c_{A\text{gas}}}{H} \]  

(5.6)
\[
\frac{\partial c_A}{\partial x'} \bigg|_{x'=0} = 0 \quad (5.7)
\]

\(x = 0\) represents the inside boundary of the biofilm, away from the gas.

In order to solve the second order partial differential equation, Orthogonal Collocation was used to approximate the concentration profile in the biofilm for the non-steady state model, following the approach by Villadsen and Stewart [178]. This approach might need more time to develop but saves computational time significantly in comparison with a finite difference scheme. The resulting ordinary differential equations are solved with Gear’s algorithm as implemented in Matlab.

The nonsteady-state model was run twice for every set of variables, once with zero initial concentration in the biofilm, and once with equilibrium concentration in the biofilm.

The steady-state method is based on Equation (5.5) with the time derivative set equal to zero:

\[
\frac{D_A}{L^2} \frac{\partial^2 c_A}{\partial x'^2} - \frac{V_{\text{max}} \cdot c_A}{K_s + c_A + \frac{S}{K_I}} \cdot \rho_{\text{bio}} = 0 \quad (5.8)
\]

which is solved with a Runge-Kutta method with adaptive step size, with the initial condition of equation (5.7). As a second initial condition, the equation is solved for a range of initial concentrations (i.e., concentrations at the inside boundary of the biofilm). Next, the outside pollutant concentration is plotted versus inside pollutant concentration. If multiple inside concentrations lead to the same outside concentration, this is indicative of multiple steady states. These steady states are confirmed with calculations with the non-steady state model as described above. The two models predict the same steady states.
within 1% when 7 collocation points are used in the nonsteady-state model, and within 0.02% when 12 collocation points are used, confirming that the algorithms used are valid.

The Buckingham π theorem was used for dimension analysis of the physical variables in order to determine the parameter space where two steady states can occur:

\[ p = n - k \]  \hspace{1cm} (5.9)

Here \( n \), \( k \) and \( p \) represent the number of variables, number of dimensions, and number of dimensionless parameters (e.g. \( \pi_1, \pi_2, \ldots, \pi_p \)), respectively. In this model, there are nine relevant variables: \( r_{\text{average}} \) (the overall degradation rate in the biofilm, \( g_{\text{substrate}} \, \text{m}^{-2} \, \text{s}^{-1} \)), \( r_{\text{external}} \) (the degradation rate assuming external conditions throughout the biofilm, \( g_{\text{substrate}} \, \text{m}^{-2} \, \text{s}^{-1} \)), \( \rho_{\text{bio}} \), \( V_{\text{max}} \), \( D_A \), \( K_s \), \( K_I \), \( c_{\text{Agas}} \, \text{H}^{-1} \), and \( L \). These include four dimensions (\( g_{\text{substrate}} \), \( g_{\text{biomass}} \), \( m \), \( s \)), leading to five dimensionless numbers. Of these, one dimensionless number (\( r_{\text{external}} \, L^{-1} \, V_{\text{max}}^{-1} \, \rho_{\text{bio}}^{-1} \)) can be written explicitly in terms of two of the other dimensionless numbers, so there is no need to explore it with computer simulations. Hence, it can be left out of the analysis and, four dimensionless numbers remain.
5.5 Results and discussion

5.5.1 Two steady states

The following dimensionless parameters were defined:

\[
\pi_1 = \frac{\text{r}_{\text{average}}}{\text{r}_{\text{external}}} 
\]

\[
\pi_2 = \frac{\rho_{\text{bio}} \cdot V_{\text{max}}}{D_A \cdot L^2 \cdot \text{c}_{\text{surface}}} = \frac{\rho_{\text{bio}} V_{\text{max}} L^2 H}{D_A C_{\text{gas}}} 
\]

\[
\pi_3 = \frac{K_m}{K_i} 
\]

\[
\pi_4 = \frac{\text{c}_{\text{surface}}}{K_m} = \frac{C_{\text{gas}}}{H K_m} 
\]

\(\pi_2, \pi_3\) and \(\pi_4\) were used to generate Figure 5.1. The kinetic parameters used in these simulations are listed in Table 5.1, where \(N\) is the number of collocation points in the biofilm and \(H\) the Henry constant.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\rho_{\text{bio}})</td>
<td>(1 \times 10^5)</td>
<td>(\text{g}_{\text{biomass}} \text{ m}^{-3})</td>
</tr>
<tr>
<td>(L)</td>
<td>100</td>
<td>(1 \times 10^{-10})</td>
</tr>
<tr>
<td>(K_m)</td>
<td>1</td>
<td>(\text{g}_{\text{Substrate}} \text{ m}^{-3})</td>
</tr>
<tr>
<td>(H)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>(N)</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>(D_A)</td>
<td>(1 \times 10^{-10})</td>
<td>(\text{g}_{\text{Substrate}} \text{ m}^2 \text{ h}^{-1})</td>
</tr>
</tbody>
</table>

\(K_i\) and \(V_{\text{max}}\) were the variables that were varied. Four values, \(K_i, 0.2, 0.5, 1 \text{ and } 2\), were used, beginning with the left upper chart, right upper chart, left lower chart and right lower
chart in Figure 5.1, corresponding with $\pi_3$ values of 5, 2, 1, and 0.5, respectively. For each change of $K_i$, $V_{\text{max}}$ was adjusted to determine the lower and the upper threshold for the occurrence of two steady states. The area between the two solid lines describes the area of two steady states, whereas the solid lines are the boundaries of the single steady state area.

The intersection of the two solid lines (henceforth called the origin) is the point where the two steady states converge to a single steady state. Larger values of $\pi_2$ (i.e., more active or thicker biofilm or lower diffusivity) and $\pi_4$ (higher concentrations) lead to larger differences between the two steady states. However, identical results in terms of the effectiveness factor ($\pi_1$) are obtained when $\pi_2$, $\pi_3$, and $\pi_4$ are kept constant while changing the individual variables. This was tested with model runs, confirming that the Buckingham $\pi$ theorem was applied correctly.

The area between the solid lines should be avoided when operating a biofilter, because a sudden change in reaction kinetics and therefore in reaction rate can occur. With change in reaction rate the removal efficiency (RE) of the biofilter will be affected as well. In particular, if the reaction rate drops, RE decreases as well.
The reason for the different steady states can be explained based on the diffusion behavior of the pollutant into the biofilm in combination with the characteristics of Haldane kinetics. Haldane kinetics is characterized by a maximum reaction rate at intermediate concentrations and low reaction rates at both low and high concentrations. If the outside surface concentration of a biofilm exceeds a certain threshold, the concentration is high enough to lead to low reaction rates near the biofilm surface. If a high concentration is present throughout the biofilm, the reactivity is low throughout the biofilm. Due to the low reactivity, diffusion limitation is not pronounced and the high concentration throughout the biofilm will be maintained over time. As long as a second concentration threshold is not exceeded, it is also possible to maintain an intermediate concentration inside the biofilm, leading to high reactivity and a pronounced diffusion limitation, which in turn maintains a
strong concentration gradient, which in turn maintains the intermediate concentration on the inside of the biofilm.

Results from the non-steady state model were validated with the steady state model. An area of two steady states can also be seen in Figure 5.2, which shows the concentration of the pollutant at the outside surface of the biofilm as a function of the concentration at the inside surface. In a certain range of surface concentration (i.e. concentration outside of the biofilm), two possible steady states occur. This range of two possible steady states is approximately 41-46 g m\(^{-3}\) at the surface. Strictly speaking, there are three steady states in this range. However, the middle steady state is unstable. In this range, a slight increase of the pollutant concentration on the inside of the biofilm leads to a pronounced decrease of the reaction rate and hence of the concentration gradient (diffusion limitation), leading to a further increase of the pollutant concentration on the inside of the biofilm. Starting from the same steady state, a slight decrease of the concentration will lead to a pronounced increase of the reaction rate and hence of the concentration gradient (diffusion limitation), further decreasing the concentration. It follows that the steady state is unstable, and will never establish spontaneously.
5.5.2 Falsified kinetics

Substrate biodegradation and inhibition in this model follow Haldane kinetics. The term falsified kinetics used here refers to the distortion of the prevailing Haldane kinetics caused by diffusion limitation. This will be illustrated using the model parameters listed in Table 5.2. These parameters are representative of toluene biofiltration Li et al. [122] and De Visscher et al [169], but with an H value of 1 so that the kinetic parameters actually reflect gas-phase values.

Figure 5.2: Concentration at the pollutant at the outside surface of the biofilm as a function of the inside concentration. Two stable steady states are predicted.
Table 5.2: Non-steady state parameters for falsified kinetics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho_{\text{bio}}$</td>
<td>$1 \times 10^5$</td>
<td>$\text{g}_{\text{biomass}} \text{ m}^{-3}$</td>
</tr>
<tr>
<td>$V_{\text{max}}$</td>
<td>0.28</td>
<td>$\text{g}<em>{\text{Substrate}} \text{ g}</em>{\text{dw}} \text{ Substrate degrading biomass}^{-1} \text{ h}^{-1}$</td>
</tr>
<tr>
<td>$K_m$</td>
<td>4.5</td>
<td>$\text{g}_{\text{Substrate}} \text{ m}^{-3}$</td>
</tr>
<tr>
<td>$K_I$</td>
<td>2.66</td>
<td>$\text{g}_{\text{Substrate}} \text{ m}^{-3}$</td>
</tr>
<tr>
<td>$N$</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>$D_A$</td>
<td>$1.8 \times 10^{-10}$</td>
<td>$\text{g}_{\text{Substrate}} \text{ m}^2 \text{ h}^{-1}$</td>
</tr>
<tr>
<td>$H$</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

In Figure 5.3, the overall reaction rate of pollutant in the biofilm is shown as a function of the inlet concentration. It can be seen that with increasing biofilm thickness the predicted kinetic behavior is changing. For the intrinsic kinetics, the model describes Haldane kinetics, as was used in the model. Here it means that there is no diffusion limitation. A very similar prediction is made at a biofilm thickness of 10 µm. In this case, even though a small biofilm exists, diffusion limitation is negligible. With increasing biofilm thickness, diffusion limitation has more influence on reaction kinetics, as can be seen for a biofilm thickness of 50 µm and 100 µm. At 100 µm the overall kinetics is more similar to Michaelis-Menten kinetics as opposed to Haldane kinetics, which can be explained by diffusion limitation. If an experimental determination of kinetic parameters is conducted under these conditions, the apparent kinetics looks like Michaelis-Menten kinetics but actually is Haldane kinetics with diffusion limitation. The apparent (falsified) kinetic parameters best fitting the diffusion-limited kinetics up to 46 g m$^{-3}$ in the presence of a 100 µm biofilm are $V_{\text{max}} = 0.0516$ $\text{g}_{\text{substrate}} \text{ g}_{\text{dw}}^{-1} \text{ h}^{-1}$, and $K_m = 7.86$ g m$^{-3}$. The fit between the falsified kinetics and the diffusion-limited simulation results in this concentration range is 1.1% (standard deviation).
Furthermore, the model predicts a sudden drop in reaction rate at a biofilm thickness of 100 µm and increasing outside concentration, at about 46 g m\(^{-3}\) toluene concentration, shown in Figure 5.3 with the solid line. When the outside concentration is decreased, starting from a high outside concentration, the reaction rate as a function of concentration deviates from the initial one, indicated with the dash line in Figure 5.3. This confirms the area of two different steady states during biofilter operation, as aforementioned.

![Figure 5.3: Falsified kinetics – Simulation of various biofilms thicknesses with Haldane kinetics](image)

**Figure 5.3: Falsified kinetics – Simulation of various biofilms thicknesses with Haldane kinetics**
The occurrence of two operational stages with different reaction rates, as shown in Figure 5.3, lead to two steady-state concentration profiles along the biofilm, as a result of diffusion limitation. A diminishingly small change of biofilm surface concentration results in the development of two significantly differing diffusion profiles in the biofilm, computed with the steady state model and shown in Figure 5.4. One profile reaches a higher concentration at the inside of the biofilm than the other and consequently displays lower activity. In turn with a higher activity, a lower concentration is present in the biofilm.

The two steady states demonstrated here result from a different mechanism than observed in past simulations. In well-mixed liquid systems the dilution of fresh influent by a dilute solution prevents the substrate concentration from reaching inhibitively high values. In this study, diffusion limitation is what maintains the low substrate concentration needed to avoid inhibition. In quorum sensing simulations, no inhibition mechanism is assumed. Instead, the diffusion through the bacterial membrane cell dilutes the signal molecule, preventing it from reaching concentrations that initiate a runaway autocatalytic process. Close proximity of bacteria lowers the flux of signal molecules through the membrane, triggering the quorum-sensing switch.
Figure 5.4: Diffusion profile in a biofilm as a function of biofilm depth showing two different steady states.

Figure 5.3 (see Table 5.2 for model parameters) explains several aspects of biofiltration, such as the need to start up a biofilter at low concentration, as the activity is highest at low concentration when the biofilm is thin. As the biofilms grow, the biofilter grows more robust to high pollutant concentrations. However, mature biofilters tend to show highly variable pollutant elimination capacities, which could be the result of alternating steady states. The parameter space for multiple steady states tends to be confined, e.g., existing only in a narrow range of biofilm thicknesses. However, sloughing of a biofilm can cause a range of biofilm thicknesses, and a thickness that is variable in time at any given location. Hence, it is likely that, at sufficiently high pollutant concentrations, parts of a biofilter are at a state of bifurcation at any given time, triggering jumps in the elimination capacity as a result of pollutant concentration fluctuations and/or sloughing.
5.6 Conclusions

The two simulation models presented here predict that kinetic bifurcation in a biofilm under aerobic conditions can occur when biodegradation rate follows Haldane kinetics (substrate limitation). One model is steady state and was used to explore the parameter space for multiple steady states. The other model is nonsteady state and allows an exploration of the initial conditions that lead to each steady state. Both models include diffusion limitation, which is a crucial element in the development of kinetic bifurcation in biofilms. The non-steady state model calculates the concentration profile in the biofilm by Orthogonal Collocation. Strictly speaking three steady states were predicted, but the intermediate state is unstable and very concentration sensitive. The emergence of the two stable steady states occur under: 1) a limiting parameter space, 2) constant external pollutant concentration exceeding or undershooting a certain threshold, and 3) Haldane kinetics.

With both models, apparent reaction rates, effectiveness factors, and concentration profiles in the biofilm can be computed. Also, the concentration as a function of biofilm depth can be determined. In addition, falsified kinetics is revealed in terms of the distortion of prevailing Haldane kinetics, which means that with increasing biofilm thickness, diffusion limitation causes the overall kinetics to resemble a Michaelis-Menten-like pattern. The occurrence of two steady states potentially leads to a sudden drop in reaction rate and consequently decreases the removal efficiency of the operating biofilter. Furthermore, distortion of apparent kinetics can result in inappropriately determined kinetic parameters.
6 Steady state stability in a toluene biodegrading biofilter: Experimental and numerical study

6.1 Abstract

The occurrence of different steady states in a toluene biodegrading biofilter was explored experimentally and numerically. Experimental results indicated a drastic alteration in steady state at an inlet concentrations change from 7.706 to 8.502 g m$^{-3}$, corresponding to a significant jump in the removal efficiency (RE) from 88 to 46%. A biofilter model that includes nitrogen and biomass dynamics was used to describe the experimental biofilter. Predicted results matched the experimental biofilter performance well, but the timing of the outlet concentration jump was not reproduced exactly. A sudden decline of 47% in RE was predicted at an inlet concentration change from 8.502 to 9.257 g m$^{-3}$. The experimental relationship between inlet and outlet toluene concentration, which showed a jump at 7.706 g m$^{-3}$ inlet concentration, was reproduced accurately by a model that assumes gradual toluene inlet concentration increase of 0.272 g m$^{-3}$ per day. Although there was variation between experimental and simulated results, a clear confirmation of the jump from one steady state to another was found. Investigation of the modeled concentration profile in the biofilm confirmed the transition from a diffusion-limited, high-activity state to a non-diffusion-limited, low activity state. In order to further validate the accuracy and applicability of the developed model, a different set of biofilter experiments were used and simulations were conducted. These results demonstrate the ability of the model to predict the overall trend of a biofilter.
6.2 Introduction

Gaseous emissions from various industrial processes need to be treated before they are released into the environment because of their harmful effect on humans, animals and the environment. Waste gas streams potentially contain hazardous substances such as volatile organic compounds (VOCs) or odorous compounds. The oil and gas, chemical and pharmaceutical industries are anthropogenic sources of such VOCs. The need to comply with stringent legislation can pose a challenge, because any waste gas treatment system must be reliable, sustainable and efficient. Physical and chemical methods are applied in industry but are often associated with disadvantages such as operational costs as well as the generation of undesirable by-products compared to their biological counterpart [68,157,159,179]. The treatment of polluted air by means of biological techniques is known and well-established in the form of a biofilter. Here, microbes degrade the compound of interest and produce water, carbon dioxide and heat [157,179].

A biofilter simulation enables the prediction of the behavior and efficiency of a system and is a substantial part of biological air pollution control research in recent decades. It is an auxiliary method to aid biofilter designers and manufacturers, as they link biological growth kinetics to reaction engineering. One of the first models was developed in 1983 by Ottengraf and Van Den Oever [117] in a study that also comprised experimental trials. Experimental and simulation results were in good agreement in terms of calculation of $\mu_{\text{max}}$ and the assumed zero-order reaction rate. Later, the development of a steady state model was conducted [118], where methanol degradation was assumed to follow a Haldane-like kinetics and oxygen a Monod-like kinetics. Also, a constant biomass content in the biofilter
was assumed in this model. Over the years, more sophisticated biofilter models were developed, incorporating a variety of parameters. This development helps to improve the accuracy of predicting an operating biofilter. A biofilter treating an air stream containing benzene has been experimentally and numerically studied [31]. The developed models are in good agreement with the experimental results. However, differences were revealed at high loading rates and low EBRT, indicating the implementation of the distribution of the active biomass density along the biofilter. In addition to biodegradation performance, biofilter models can predict other features as well. For example, the experimental and computational evaluation of heat generation in a trickling biofilter treating toluene has been studied [180]. This model shows the capability to predict the heat and mass transfer and, hence, the temperature trend between the inlet and the outlet of the biofilter. The biomass accumulation in a toluene treating biofilter at high loads was simulated by Dorado et al. [181]. The model incorporated advection, absorption, adsorption, diffusion, biodegradation and biomass growth. The verification against experimental results was satisfactory. A binary VOC mixture was treated in a biofilter and described by a mathematical model in [182]. Potential kinetic interaction between the pollutant, biomass differentiation, and oxygen availability for biodegradation were considered. Regarding the biomass differentiation, it was assumed that the packing material is not necessarily entirely covered with biofilm and hence forms patches of biofilm on the surfaces of the packing material. Therefore, two cases were considered: 1) both VOCs can be degraded by a patch of biofilm (data from a benzene/toluene biofilter), or 2) only one VOC can be degraded by a patch of biofilm (data from ethanol/butanol biofilter). It was shown that the agreement between experimental data and model prediction was better for the benzene/toluene case. With both
models good agreement between model predictions and experimental data were found. Shareefdeen [183] demonstrated the important role of a mathematical model, predicting odor removal performance in a biofilter to facilitate the design and evaluation of operational conditions of a rendering process. In this work, the models developed by Ottengraf and van den Oever (zero-order diffusion limited, zero-order reaction limited, and first-order) were extended to describe the prediction of odor removal efficiency. The first-order model fit the pilot plant data most accurately, with a correlation coefficient of 0.94, and was used in designing a full-scale biofilter system.

Despite the continuous development of computer simulation models, uncertainties are still present. For example, the knowledge of different steady states and their impact is well established in other biological applications as indicated in Chapter 5. Hence, it is reasonable to expect a similar behavior in a biological system such as a biofilter. The occurrence of multiple steady states in a biofilter is numerically shown in Chapter 5. However, the Chapter only considers a biofilm, not a whole biofilter. Hence, the further development of such a computer simulation and exploration of possible effects would aid biofilter designers and operators. In addition, an experimental verification to reinforce the theory about multiple steady states in a VOC degrading biofilter would be beneficial. Therefore, the computational and experimental investigation of multiple steady states are addressed in order to fill that gap in knowledge.
6.3 Materials and methods

6.3.1 Microorganism and filter media

For the filter bed, a sterilized mixture of compost and wood chips (vol% 80/20) was used. In Table 6.1, the filter bed analysis is shown. Analysis were conducted by AGAT Laboratories. All chemicals used, benzene, toluene, ethylbenzene, m-xylene and solids for the cultivation medium, were obtained from Sigma Aldrich. An adapted liquid BH-medium [123] was used as a growth medium and contained 1 g L\(^{-1}\) KH\(_2\)PO\(_4\), 1 g L\(^{-1}\) Na\(_2\)HPO\(_4\), 0.5 g L\(^{-1}\) NH\(_4\)NO\(_3\), 0.002 g L\(^{-1}\) FeCl\(_3\), 0.002 g L\(^{-1}\) MnSO\(_4\)·2H\(_2\)O, 0.2 g L\(^{-1}\) MgSO\(_4\)·7H\(_2\)O and 0.02 g L\(^{-1}\) CaCl·2H\(_2\)O. The medium was sterilized before used.

As a single carbon source, toluene was used. The growth medium was used to culture a toluene degrader, *Nocardia* sp. [134]. In order to introduce additional microorganisms into the inoculant, an air stream was piped into the serum bottles. After 3 days, plating of cells was conducted, and standard procedures were followed [135]. Results indicated one microbe able to degrade toluene, the *Nocardia* sp. of the pure inoculant. Two other bacterial strains were found. These other two bacteria were tested on their ability to biodegrade toluene as a single carbon source, and were not able to biodegrade toluene. Therefore, it is assumed that *Nocardia* sp. is the only BTEX-degrading microbe in the mixture. No further characterization of microbes was conducted. *Nocardia* sp. as BTEX-degrading and two non-BTEX degrading microbes were cultivated under ambient temperature (21-22°C) in serum bottles sealed with a butyl rubber septum and aluminum crimp se
Table 6.1: Filterbed properties

<table>
<thead>
<tr>
<th>Filterbed properties</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.30</td>
<td>-</td>
</tr>
<tr>
<td>Particle Size Distribution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2000-50 μm)</td>
<td>44</td>
<td>%</td>
</tr>
<tr>
<td>(50-2 μm)</td>
<td>37</td>
<td>%</td>
</tr>
<tr>
<td>(&lt;2 μm)</td>
<td>19</td>
<td>%</td>
</tr>
<tr>
<td>NO$_3$ - N and NO$_2$-N</td>
<td>916</td>
<td>mg kg$^{-1}$</td>
</tr>
<tr>
<td>Total Nitrogen</td>
<td>15900</td>
<td>mg kg$^{-1}$</td>
</tr>
<tr>
<td>PO$_4$-P</td>
<td>194</td>
<td>mg kg$^{-1}$</td>
</tr>
<tr>
<td>Organic Matter</td>
<td>29.50</td>
<td>%</td>
</tr>
</tbody>
</table>

6.3.2 Biofilter set-up and experimental conditions

The BTEX biofiltration experiments were carried out in a lab-scale biofilter with an inoculation volume of 100 mL (OD$_{650}$ around 0.2). The column was made of polycarbonate with a total height of 63 cm and an internal diameter of 10 cm. At the bottom, glass beads at a height of 5 cm and a 1 cm perforated Plexiglas plate were used to evenly distribute the inlet gas stream. Sampling ports to measure gas samples were centered at the top and bottom of the column and sealed with GC septa (0.95 cm diameter). The biofilter was filled with the filter media to a height of 25 cm and placed in a fume hood under ambient temperatures of 21-22°C. A schematic of the experimental set-up is shown in Figure 6.1. An air pump (pond master Ap-40) was used to generate one air stream, which was split and sent through two gas washing bottles, one filled with tap water and one filled with the
contaminant. Subsequently, the two gas flows were combined and mixed in an empty gas-washing bottle before being introduced into the biofilter. The flow was controlled by two rotameters (Cole-Parmer) located after the gas flow split, and total flow rate was measured (TI-400) before the sample port and inlet.

Figure 6.1: Schematic of the biofilter set-up: 1) air pump, 2) rotameters, 3) water vessel, 4) toluene vessel, 5) mixing vessel, 6) flow meter and 7) biofilter
6.3.3 Biofilter performance parameters

The performance of a biofilter is commonly described by the removal efficiency (RE) and the elimination capacity (EC). They depend on the inlet loading rate (ILR), and the empty-bed residence time (EBRT). They are defined as follows:

\[ RE = \frac{C_i - C_o}{C_i} \times 100 \]  \quad (2.6)

\[ ILR = \frac{Q \cdot C_i}{V} \]  \quad (2.5)

\[ EC = \frac{Q \cdot (C_i - C_o)}{V} \]  \quad (2.7)

\[ EBRT = \frac{V}{Q} \]  \quad (2.1)

where \( C_i \), \( C_o \), \( Q \), and \( V \) represent the inlet concentration [g m\(^{-3}\)], outlet concentration [g m\(^{-3}\)], flow rate [m\(^{3}\) h\(^{-1}\)] and biofilter volume [m\(^{3}\)], respectively.

6.3.4 Biofilter operation

An air stream contaminated with toluene was treated under different operational conditions. An EBRT of 4.5 min was maintained and a stepwise increase of the inlet concentration was conducted. Adsorption test for single toluene was conducted and indicated no adsorption due to the sterilized filter packing or column. The corresponding Figure is shown in appendix A. The operational parameters are shown in Table 6.2.
6.3.5 Analytical methods

A SGE 250 µL gastight syringe was used to draw and inject 200 µL of gas sample from the gas sampling ports and into the analyzer. The gas samples were analyzed with a gas chromatograph (GC-2014, Shimadzu) equipped with an FID and Rtx®-Wax capillary column (30 m x 0.53 mm x 1 µm). The injector and detector temperatures were set at 250°C. The oven temperature for toluene was 80°C. Helium was used as a carrier gas.

6.4 Model description

The occurrence of multiple steady states is well known and established in different biological and chemical processes. However, in biofiltration this phenomenon is not well explored and understood. A numerical approach was conducted in Chapter 5 and the presence of different steady states was established inside a biofilm, considering no other back-mixing mechanisms. It was also established that a change in steady state can lead to a sudden decrease in RE. To further investigate the possible steady states in a biofilter, the present model was developed. It enables the prediction of an actual biofilter. The following assumptions were made for the model:

1. Gas phase flow is assumed to behave in plug flow pattern. Thus, axial dispersion is neglected.
2. Due to low gas phase concentration, the gas-biofilm equilibrium is described by Henry’s law.
3. A planar geometry of the biofilm is assumed.
4. Haldane kinetics is assumed to describe substrate biodegradation and substrate inhibition.

5. Oxygen is not considered as a limiting factor.

6. Inorganic nitrogen cycling is considered in the model and described in [134].

6.5 Model development

Since the model developed here is a further development of a previous one, the description of diffusion, biodegradation rate and their combined mathematical treatment are identical to those in Chapter 5. In order to predict the outlet concentration of the biofilter, the packing material was divided into 25 collocation points along the biofilter height. For each collocation point, an average reaction rate, net growth rate, biofilm concentration profile, and gas phase concentration were computed. In addition, a nitrogen cycle was also considered in the model [184].

The molecular diffusion described in this model is governed by Fick’s law:

\[ J = D_A \frac{dc_A}{dx} \]  

(5.1)

where \( J \), \( D_A \), \( c_A \) and \( x \) refer to the diffusive flux of component A, the diffusion coefficient of component A [gSubstrate m\(^{-2}\) h\(^{-1}\)], the concentration of the compound A [gSubstrate m\(^{-3}\)] and the length coordinate [m] in the direction of the biofilm thickness. The biodegradation rate of toluene was assumed to follow Haldane kinetics, which considers substrate inhibition and consequently is written as the reaction rate \( r \), as follows:

\[ r = \frac{V_{max} c_A \rho_{bio}}{K_s + c_A + \frac{c_A^2}{K_I}} \]  

(5.2)
\( V_{\text{max}} \) expresses the maximum toluene degradation rate per unit weight of biomass per hour \([\text{g}_{\text{substrate g}_{\text{dw}}} - 1 \text{ h}^{-1}]\), \( K_s \) refers to the Michaelis-Menten constant \([\text{g}_{\text{substrate m}^3}]\), \( K_I \) represents the inhibition constant \([\text{g}_{\text{substrate m}^3}]\) and \( \rho_{\text{bio}} \) reflects the biomass density of microorganisms in the biofilm \([\text{g}_{\text{dw m}^3}]\).

In order to calculate the concentration profile in the biofilm, diffusion and reaction rate, equations (6.5) and (6.6), were linked together, considering the biofilm thickness \( L \) [m], the distance coordinate in the biofilm \( x \) [m] and a dimensionless distance coordinate in the biofilm \( x' \) using a material balance, which leads to the following expression.

\[
\frac{\partial c_{\text{A}_{\text{biofilm}}}}{\partial t} = \frac{D_A}{L^2} \frac{\partial^2 c_{\text{A}}}{\partial x'^2} - \frac{V_{\text{max}} c_{\text{A}}}{K_s + c_{\text{A}} + \frac{S}{K_I}} \rho_{\text{bio}} \tag{5.5}
\]

To solve the above equation, the following boundary conditions were used:

\[
c_{\text{A}}\bigg|_{x' = 1} = \frac{c_{\text{Agas}}}{H} \tag{5.6}
\]

\[
\frac{\partial c_{\text{A}}}{\partial x'}\bigg|_{x' = 0} = 0 \tag{5.7}
\]

where the inside boundary of the biofilm away from the gas is represented with \( x = 0 \). The partial differential equation in equation (6.7), which is first-order in time, and second-order in space, was solved by Orthogonal Collocation to approximate the concentration profile in the biofilm and refers to [178]. Next, the concentration in the gas phase in each collocation point, was computed by equating the transfer of toluene in the gas phase towards the biofilm to the integrated toluene biodegradation in the biofilm.

\[
\frac{dc_{\text{Agas}}}{dt} = - \frac{\partial c_{\text{Agas}}}{\partial t} - \rho_{\text{bulk}} A L \bar{r} \tag{6.1}
\]
Where

\[
\bar{r} = \int_0^1 V_{\text{max}} \frac{C_{\text{biofilm}} \rho_{\text{bio}}}{K_c C_{\text{biofilm}}^2 + \frac{C_{\text{biofilm}}^2}{K_c}} \, dx' 
\]  

(6.2)

Here \(v\) expresses the superficial velocity [m h\(^{-1}\)] and \(A\) the specific surface area of the biofilm [m\(^2\) kg\(^{-1}\)].

To account for the biofilm growth, the net growth rate of the microorganism needs to be considered. As mentioned above, the nitrogen cycle is considered in this model and a part of it is expressed in the following equation:

\[
\mu = \left( \frac{\mu_{\text{max}} C_{\text{Agas}}}{K_s + C_{\text{Agas}} + \frac{C_{\text{Agas}}^2}{K_I}} \right) \left( \frac{N_{\text{inorg}}}{K_{N_{\text{Nitrogen}}} + N_{\text{inorg}}} \right) 
\]  

(6.3)

\[\mu_{\text{net}} = \mu - a\]  

(2.49)

where, \(\mu_{\text{max}}, \mu_{\text{net}}, N_{\text{inorg}}\) and \(K_{N_{\text{Nitrogen}}},\) expressing the maximum growth rate [h\(^{-1}\)], the net growth rate [h\(^{-1}\)], inorganic nitrogen concentration of the packing material [g\(_N\) kg\(_{\text{compost}_{dw}}\) \(^{-1}\)], and the Michaelis-Menten constant for nitrogen utilization [g\(_N\) L\(_{H_2O}\)], respectively. The growth of the biofilm as a function of time is described by

\[
\frac{dx}{dt} = \mu_{\text{net}} X
\]  

(2.48)

where \(X\) is the biomass concentration [g\(_{dw_{\text{biomass}}}\) kg\(_{\text{compost}}\) \(^{-1}\)].

The consumption of inorganic nitrogen is calculated as follows:

\[
r_N = \bar{r} \int \rho_N \rho_{\text{bio}} A \, L
\]  

(6.4)
where $f_N$ is the nitrogen fraction of the microorganisms, and $\bar{\mu}$ is the average growth rate over the biofilm, calculated in a manner similar to $\bar{r}$. The dynamics of inorganic nitrogen is calculated as follows and shown in [184]:

$$\frac{dN_{\text{inorg}}}{dt} = k_{\text{minN}} N_{\text{org}} - k_{\text{uptakeN}} N_{\text{inorg}} - rN$$

(6.5)

where $N_{\text{inorg}}$, $k_{\text{minN}}$ and $k_{\text{uptakeN}}$ represent the inorganic nitrogen content in the packing material [$gN$ kg$_{dw}^{-1}$], the nitrogen mineralization constant [h$^{-1}$], and the nitrogen uptake rate [h$^{-1}$], respectively.

### 6.6 Results and discussion

#### 6.6.1 Experimental data

In Figure 6.2 the inlet and corresponding outlet concentrations of the experimental trial, are displayed in order to evaluate the occurrence of two steady states. The outlet concentration barely changes, although the inlet concentration slowly increases until day 31. Here the RE declines from 99% to 88% as the inlet load increases by 4.88 g m$^{-3}$ (increase from 2.886 to 7.706 g m$^{-3}$). It should be noted that the inlet concentration was adjusted and increased prior to day 31, but the actual measurement was taken on day 31. This is indicated in Figure 6.2, 6.3 and 6.4 with a theoretical calculated inlet concentration depicted as a rhomboid. The next increase of the inlet concentration was by 0.796 g m$^{-3}$ (to a value of 8.502 g m$^{-3}$), which led to a steep increase of the outlet concentration. This corresponds to a decrease of RE from 88% to 46%. Such a significant decline in RE for a small increase in inlet concentration could indicate that a boundary of a stable steady state region has been crossed.
The occurrence of multiple steady states in an aerobic biofilm treating a VOC was discussed and numerically elucidated in Chapter 5. A change in steady state could be explained by substrate degradation and substrate inhibition following Haldane kinetics and the diffusion behavior of the pollutant into the biofilm. Distinctive for Haldane kinetics is that low reaction rates occur at low and high concentrations and high reaction rates occur at medium concentrations. With regard to the conducted experiments, the transition from high to low reaction rates is in the range of 7.706 g m$^{-3}$ to 8.502 g m$^{-3}$. Furthermore, diffusion-limitations are an important factor as well. When medium range concentrations are present at the surface of the biofilm it is possible to maintain such concentrations throughout the biofilm, leading to a high reactivity and pronounced diffusion limitation. Hence, a significant concentration gradient will be upheld in the biofilm and well consequently maintain a medium range concentration at the inside of the biofilm. On the other hand, if a high enough concentration is present at the surface of the biofilm, reaction rates near the surface are low. Thus, a high concentration can develop throughout the biofilm and therefore result in low reactivity. In this case, diffusion limitation is not pronounced.
Figure 6.2: Experimental results of toluene biofiltration in conditions designed to yield two steady states (EBRT = 4.5 min). The single data point depicted as a rhomboid, represents the theoretical increase in the biofilter inlet after increasing the inlet concentration – the actual measurement at the inlet was carried out at a later day.
6.6.2 Fitting computer simulation to experimental data – steady states

The computer simulation developed here was used to predict the outlet concentrations based on obtained inlet concentrations of the experimental trial. To optimize the model, parameters $A$ and $k_{\text{min}}$ were used as adjustable variables. The values of $V_{\text{max}}$, $K_{m}$, and $K_{I}$ were taken from Chapter 3. The remaining parameters were based on literature values. Used model parameters are displayed in Table 6.2 and the simulation results and experimental data are shown in Figure 6.3.

<table>
<thead>
<tr>
<th>Used model parameters</th>
<th>experiment 2 steady states</th>
<th>steady increase of inlet concentration</th>
<th>biofilter experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoUlocation points (biofilm)</td>
<td>10</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Henry's constant (H)</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Initial biomass ($X_{0}$)</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003 $\text{g/m}_\text{biomass}$</td>
</tr>
<tr>
<td>Specific surface area of biofilm (A)</td>
<td>0.95</td>
<td>1.1</td>
<td>0.2375 $\text{m}^2/\text{kg}$</td>
</tr>
<tr>
<td>Yield (Y)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5 $\text{g/g}$</td>
</tr>
<tr>
<td>$\mu_{\text{max}}$</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1 $\text{h}^{-1}$</td>
</tr>
<tr>
<td>$V_{\text{max}}$</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3 $\text{g g}^{-1} \text{h}^{-1}$</td>
</tr>
<tr>
<td>Decay rate ($k_{d}$)</td>
<td>0.0014</td>
<td>0.0014</td>
<td>0.0014 $\text{h}^{-1}$</td>
</tr>
<tr>
<td>Superficial velocity (us)</td>
<td>3.63</td>
<td>3.63</td>
<td>3.63 $\text{m h}^{-1}$</td>
</tr>
<tr>
<td>Organic nitrogen content ($N_{\text{org}}$)</td>
<td>14.98</td>
<td>14.98</td>
<td>14.98 $\text{g N/g compost}$</td>
</tr>
<tr>
<td>Half-saturation constant $K_{m}$</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05 $\text{g m}^{-3}$</td>
</tr>
<tr>
<td>Inhibition constant $K_{I}$</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7 $\text{g m}^{-3}$</td>
</tr>
<tr>
<td>Nitrogen uptake rate constant ($k_{\text{uptake}}$)</td>
<td>0.0022</td>
<td>0.0022</td>
<td>0.0022 $\text{h}^{-1}$</td>
</tr>
<tr>
<td>Nitrogen mineralization constant ($k_{\text{min}}$)</td>
<td>0.00007</td>
<td>0.00007</td>
<td>0.00007 $\text{h}^{-1}$</td>
</tr>
<tr>
<td>Michaelis-Menten constant for nitrogen ($K_{S}$)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5 $\text{g L}^{-1}$</td>
</tr>
<tr>
<td>Mass fraction of nitrogen in toluene degrading biomass ($f_{N}$)</td>
<td>0.126</td>
<td>0.126</td>
<td>0.126 $\text{g N/g biomass}$</td>
</tr>
<tr>
<td>Porosity of the biofilter ($\varepsilon$)</td>
<td>0.55</td>
<td>0.55</td>
<td>0.55 $\text{m}^2/\text{kg}$</td>
</tr>
<tr>
<td>Diffusivity in biofilm (D)</td>
<td>1.5E-10</td>
<td>1.5E-11</td>
<td>1.5E-12 $\text{m}^2\text{s}^{-1}$</td>
</tr>
<tr>
<td>Density of bulk ($\rho_{\text{bulk}}$)</td>
<td>555</td>
<td>555</td>
<td>306 $\text{kg compost/m}^3$</td>
</tr>
<tr>
<td>Density of biofilm ($\rho_{\text{bio}}$)</td>
<td>1.00E+05</td>
<td>1.00E+05</td>
<td>1.00E+05 $\text{g m}^{-3}$</td>
</tr>
</tbody>
</table>
The model prediction and the experimental results are in good agreement until day 23. After that day, the predicted outlet concentration suddenly rose and declined over a period of 8 days. It should be noted that the inlet concentration during the experiment was adjusted on day 24 but the actual measurement was taken on day 31. This is indicated in Figure 6.3, with the expected inlet concentration after adjustment depicted as a rhomboid. That increase of the inlet concentration caused the predicted sudden rise at the outlet. The following predicted decline of outlet concentration is possible due to the adaption of the system to the high stepwise increase of the inlet concentration (biofilm growth). During the next time period (day 31 to 37) the experimental trial indicates a change in steady states, whereas the model prediction indicates such a change between day 37 and 38, at a concentration change from $8.502 \text{ g m}^{-3}$ to $9.257 \text{ g m}^{-3}$. This corresponds to a 47% decline in RE. Between day 37 and 40 the maximum achieved decline in RE is 72%. Although the change in steady state is not predicted in the same time period and concentration range, an indication of change can be seen. A further adjustment of model parameters could possibly increase the accuracy of the model.
Figure 6.3: Experimental results and model prediction of toluene biofiltration (EBRT = 4.5 min). The single data point depicted as a rhomboid, represents the theoretical increase in the biofilter inlet after increasing the inlet concentration – the actual measurement at the inlet was carried out at a later day.

Nevertheless, to show the change in steady state and the significance of the model, a second model run was carried out, where the inlet concentration was steadily increased with uniform increments of 0.272 g m$^{-3}$ per day. The results are shown in Figure 6.4, overlaid with the experimental data. To optimize the model fit, the surface area (A) was increased slightly from 0.95 m$^2$ kg$^{-1}$ to 1.1 m$^2$ kg$^{-1}$.
As depicted, the predicted change in outlet concentration and the corresponding RE are barely changing until an inlet load of 7.909 g m\(^{-3}\) is reached at day 29. Here the RE declines from 99.93\% to 43.79\% on day 30 at a concentration change of 0.272 g m\(^{-3}\). Another stepwise increase of inlet concentration led to a further decrease of RE to 40.39\%. Henceforth a continuous and slow decrease of RE can be observed based on the increasing outlet concentration. When aforementioned sudden decrease of RE (from 99.93\% to 43.79\%) is compared to the experimentally obtained results, a similar behavior can be observed. Which means at a small inlet concentration change of 0.796 g m\(^{-3}\) at the experiment the RE decreased from 88.269\% to 46.365\%. This behavior is indicated in Figure 6.4 on day 31 and day 37. Consequently in both cases, simulation and experimental
results, a change in steady state occur at similar concentration. The modeled jump is
sharper than the observed jump. This is because the model assumes cross-sectionally
uniform biofilm thickness, whereas the actual biofilm will not be uniform within a cross-
section. The inlet concentration before the jump was 7.909 g m\(^{-3}\) for the simulation and
7.706 g m\(^{-3}\) for the experiment, and the inlet concentration after the observed jump were
8.181 g m\(^{-3}\) and 8.502 g m\(^{-3}\) for the simulation and experiment, respectively.

Figure 6.5 is included to better illustrate the similarity between the experimental data and
the simulation shown in Figure 6.4. In Figure 6.5 the outlet concentration is plotted versus
inlet concentration. A very good agreement is obtained. In this Figure, a significant
increase in the outlet concentration is observed at a small change in inlet concentration.

![Outlet concentration versus inlet concentration graph](image)

**Figure 6.5:** Outlet concentration versus inlet concentration of experimental trial and simulation with steady increase of inlet concentration
This indicates a jump from a high activity steady state to a low activity steady state. The observed behavior should not be confused with a collapse of the biofilm. A collapsing biofilm at such a low inlet concentration change would occur over a certain time, not suddenly, considering no other changing factor or inhibition.

In above simulation, the concentration in the biofilm is low when high activity is predicted and high when low activity is shown. This indicates a non-saturated and saturated biofilm and consequently results in a high and low activity as aforementioned and elucidated in Chapter 5. This is illustrated Figure 6.6 were the concentration in each collocation point is shown at day 37 (888 hours) and day 37.04 (889 hours) simulating the experimental conditions. The concentration is plotted as a function of distance from the solid surface of the packing material to the surface of the biofilm. Were a single data point represents one collocation point. It can be seen, that at day 37 the concentration sharply declines towards the insight of the biofilm, which indicates a non-saturated biofilm and high activity. One hour later, at day 37.04 the concentration in the biofilm is more stable, indicating a saturated biofilm and less activity.
Figure 6.6: Predicted concentration in the biofilm by using the experimental inlet concentration. Showing a sudden change from non-saturated to saturated biofilm.

### 6.6.3 Fitting computer simulation to experimental data – biofilter

To further validate the accuracy and applicability of the computer simulation, it was verified against another set of biofilter experiments conducted in Chapter 3. In this experiment the inlet concentration was increased stepwise and held at each stage until a pseudo-steady state was reached based on the obtained steady RE. In Figure 6.7 the predicted outlet and experimental inlet and outlet concentrations are shown and the used model parameters are listed in Table 6.2. To get a good fit between the model and the data, the specific surface area was reduced by a factor 4. This corresponds with increasing the packing size from about 6mm to about 25mm (assuming spherical particles and a solid
density of 1000 kg m\(^{-3}\)). These are reasonable values, and the increase was expected because the straw used as a bulking agent in Chapter 3 did not sustain the structure of the biofilter as well as the wood chips used here.

The model follows the experimental data well until day 36. After that, the model underpredicts the outlet concentration. It is hypothesized that settling of the biofilter reduced the biofilm specific surface area exposed to the gas phase. Some slight settling was observed in this biofilter, unlike the biofilter of the data in Figure 6.2. The reduced specific surface area reduced the biofilm area in the biofilter. The simulations indicate that the biofilm is strongly diffusion-limited in this biofilter, so a direct proportionality between biofilm area and activity is expected. The assumption of proportionality between activity to specific surface area is consistent with [69], who found that maximum EC decreased with increasing particle size, but increases with increasing specific surface area. This implies, that at that point in time the biofilm surface area has a more pronounced impact on the RE then the activity of the biofilm.
Figure 6.7: Experimental inlet and outlet concentration and predicted outlet concentration.
6.7 Conclusion

In the present study the occurrence of a jump from high-activity steady state to a low-activity steady state was experimentally and numerically explored. Both results show that such a jump does indeed occur, however at a slightly different timing. A decline of 47% in RE was predicted at an inlet concentration of 8.502 g m$^{-3}$, which compared well with the 42% decline experimentally determined at an inlet concentration of 7.706 g m$^{-3}$. When the inlet concentration was increased gradually at constant rate in the model (0.272 g m$^{-3}$), the relationship between inlet and outlet concentration can be reproduced very well. In addition, the model was compared with a toluene degrading biofilter used in a previous study. Results showed an overall prediction of the outlet concentration, except at the end of the experiment, where settling may have reduced the biofilm area. An investigation of modeled toluene concentration profile in the biofilm before and after the sudden jump in RE confirmed that the cause of the jump is a transition from a diffusion-limited high-activity state to a low-activity state.
7 Conclusion and recommendations

The potential of Nocardia sp. in a mixture with two non-degrading bacteria treating single compounds of BTEX and a BTEX mixture in a biofilter, was shown for the first time. In addition, the high affinity of the microorganism to BTEX, especially at low concentration of BTEX, is revealed by the conducted biofilter experiments. Such a behavior of microorganisms used in a biofilter is exceptional. Supporting indication were found as kinetic parameters were approximated. Despite the deviation of kinetic parameters compared to literature results, the achieved REs of the biofilters are comparable or exceed efficiency, when compared to similar studies.

Experimental trials were conducted under two different EBRTs (1.5, 2.5 min) and three average inlet concentrations (0.050 g m\(^{-3}\), 0.600 g m\(^{-3}\), 1.5 g m\(^{-3}\)) for single compounds of BTEX. For an inlet loading rate (ILR) of 0.976 g m\(^{-3}\) h\(^{-1}\) and 1.697 g m\(^{-3}\) h\(^{-1}\) of toluene and ethylbenzene, REs > 93% and 95% were achieved, respectively. Also, efficiencies of 90% and higher were achieved for benzene and 87% for m-xylene. Kinetic parameters were determined in batch tests, and as aforementioned, differ from those found in literature. Furthermore, the performance of biodegrading a mixture of BTEX in an air stream with the same experimental set-up and inoculant was conducted. At lower inlet concentrations and low EBRT (1.5 min), the average maximum REs for toluene and ethylbenzene are > 90%, with an average maximum EC of 8.5 g m\(^{-3}\) h\(^{-1}\) and 1.44 g m\(^{-3}\) h\(^{-1}\), respectively. For benzene and m-xylene RE < 90% and < 35% were obtained, respectively. With an increase of EBRT to 2.5 min the RE increased accordingly and the same behavior was observed for higher inlet concentrations. Kinetic parameters were estimated and the acquired kinetic parameters are not consistent with literature findings. However, the
presented results ([Chapter 3 and 4](#)) of an engineered microbial system containing one-BTEX degrader and two non-BTEX degraders show an effective method to treat a gas stream containing single BTEX compounds or a blended of BTEX.

In addition, the occurrence of multiple steady states in a biofilter was investigated for the first time. Multiplicity of steady states in a biofilm was explored. To that effect, two independent computer models were developed to verify each results. The presented simulations predict that a jump of steady state in a biofilm under aerobic conditions, which can occur when the biodegradation rate follows Haldane kinetics and is subjected to diffusion limitation. A jump of steady state possibly leads to a decrease of removal efficiency and consequently to a decline in biofilter performance. In addition, falsified kinetics is revealed in terms of the distortion of prevailing Haldane kinetics, which means that with increasing biofilm thickness, diffusion limitation causes the overall kinetics to resemble a Michaelis-Menten-like pattern. A distortion of apparent kinetics can result in inappropriately determined kinetic parameters. The presented results ([Chapter 5](#)) are unique and lead to further investigations.

Based on the above mentioned results ([Chapter 5](#)), the non-steady state model was further developed ([Chapter 6](#)) in order to investigate the occurrence of multiple steady states in an biofilter. The comprehensive, in term of its complexity, biofilter model, predicts a jump of steady state. In addition, an experimental trial was carried out to experimentally support the obtained results. It was shown that experimental and simulation results indicate a jump of steady state. Noteworthy is the use of kinetic parameters obtained in this dissertation, did lead to a very good fit between model prediction and experimental results. However, the deviation in the experiment and simulation is likely based on the complex model and
its vast possibilities of model parameters. This makes it difficult to determine the model parameters representing the experimental trial.

Biofilter experiments (Chapters 3 and 4) show high BTEX removal efficiencies. An upscale of the biofilter could be a next step in order to obtain more data on a possible industrial application. In addition, the possible increase in efficiency based on nutrient availability should be further investigated in terms of providing nutrients through the humidified air.

The results in regards to the multiplicity of steady states (Chapters 5 and 6) representing the first step in investigating multiple steady states in a biofilter and the adverse effect, need to be further elucidated. It can be of great benefit for biofilter designers and the industry to predict a sudden drop in removal efficiency, which would be devastating for a biofilter operator. A possible way to increase the accuracy of the model is a simplification, where less variables need to be adjusted. However, with a reduction of adjustable parameters, the intervene and control possibilities are reduced. Further experiments would be beneficial to verify the concentration range were a jump from one steady state to another occur.
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9 Appendix

Appendix A: Biofilter adsorption tests
Appendix B: Batch test – adsorption test