NOVEL MEMBRANE BASED BIOLOGICAL WASTE GAS TREATMENT SYSTEM

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presented by
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Abstract

Biological waste gas treatment is an attractive method for controlling air emissions of biodegradable volatile organic compounds (VOCs). Microorganisms degrade the VOCs into harmless products, such as carbon dioxide, biomass and water. Yet, in spite of these assets, unsolved challenges remain for biological waste gas treatments in comparison to conventional physico-chemical procedures. Fluctuating loads in waste gas streams, especially from VOCs with low water solubility, often cannot be satisfactorily removed. Concentration peaks leave the reactor virtually untreated, while periods without VOCs in the waste gas lead to a reduction in the activity of the biofilm. Furthermore, bioreactors are often subject to clogging due to excessive biomass accumulation.

The objective of the present work was to design and characterize a novel system, which is able to buffer fluctuating loads of toluene as example for poorly water soluble VOCs and continuously remove and discharge inactive, excessive biomass from the reactor.

A flat sheet membrane reactor was built and investigated with an absorption process introduced as a buffering step. The VOCs and oxygen are withdrawn from the waste gas and buffered in silicon oil prior to bacterial degradation. The absorption and the biodegradation are both membrane based. Dense, 50 μm thick, poly dimethyl siloxane (PDMS) membranes featuring an integrated stainless steel grid for mechanical stability are used to separate the distinct phases. A bacterial biofilm, which degrades the buffered VOCs, develops on the membrane, separating the aqueous phase from the absorbent. The culture medium is constantly passing along the biofilm introducing shear stresses on the
surface and thereby removing excess biomass. The feeding of the VOCs and oxygen through the membrane to the base of the biofilm creates an activity distribution with active biomass at the membrane and spent biomass towards the aqueous phase. Therefore, the discharge of biomass does not interfere with the degradation performance.

Experiments were performed to investigate the effects of toluene loading rate and gas residence time on the performance of a laboratory-scale membrane bioreactor under steady-state and dynamic conditions. The surface elimination capacity was virtually independent of the gas flow rate and increased with increasing surface loads to reach a maximum elimination capacity of 0.6 g/m²h for surface loads beyond 3 g/m²h. Experiments with fluctuating inlet mass flow rates of toluene demonstrated the buffering capability of the set-up.

The biofilm was investigated with respect to the spatial distribution of bacterial activity and the composition of the microbial consortium. Oxygen concentration profiling, as well as live/dead confocal laser scanning microscopy (CFLSM) analyses of the biofilm indicated that microbial activity was limited to an 80 μm thick zone next to the membrane. Selective plate experiments showed that 50 days after inoculation the percentage of aerobic culturable cells in the biofilm, which were able to metabolize toluene, decreased to 40%. The toluene degraders consisted of a considerable fraction (between 40 and 70%) of bacteria that were different from the two originally inoculated strains *Pseudomonas putida* F1 and *Rhodococcus globerulus* PWD1.

In order to conduct sensitivity analyses and to perform scaling-up feasibility studies, a model of the process was developed. The four phases – gas, absorbent, biofilm, culture liquid – were modeled in equilibrium at their boundaries. For each phase, differential equations for the toluene and oxygen concentrations were derived (time dependent, locally one-dimensional). The model was numerically solved by applying an ODE solver using spatial discretization. The biofilm activity was modeled on a double Monod kinetic. The active biomass was distributed over the biofilm thickness according to the results of the CFLSM measurements. The sensitivity analysis of the model indicated that the removal capacity was strongly influenced by the yield coefficients $Y_{xs}$ and $Y_{XO_2}$ as well as by decreasing maximum specific growth rates $\mu_{\text{max}}$. 
Zusammenfassung


Das Ziel der hier vorliegenden Arbeit war, ein neues System zu entwickeln und charakterisieren, welches in der Lage ist, schwankende Ladungen von Toluol als Modellsubstanz für schlecht wasserlösliche VOCs zu puffern und das überschüssige, inaktive Biomasse kontinuierlich aus dem Reaktor entfernt.


Der Effekt der Toluol-Beladungsrate und der Verweilzeit des Modellgases auf die Leistung des Labormassstab-Reaktors unter konstanten und dynamischen Bedingungen wurde experimentell untersucht. Die Oberflächen-Eliminierungskapazität war weitgehend unabhängig von der Gasflussrate und stieg mit steigender Oberflächenbeladung, um eine maximale Eliminierungskapazität von 0,6 g/m²h für Beladungen kleiner als 3 g/m²h zu erreichen. Experimente mit schwankenden Toluol-Massenflüssen im Einlassgas zeigten die gute Pufferungskapazität des Reaktors.

Der Biofilm wurde hinsichtlich der örtlichen Verteilung der bakteriellen Aktivität und der Zusammensetzung des mikrobiellen Konsortiums untersucht. Das Sauerstoffkonzentrations-Profil als auch die Vitalitätsanalyse mit Hilfe der konfokalen Laserscanning-Mikroskopie demonstrierten, dass die mikrobielle Aktivität auf eine 80 μm dicke, direkt auf der Membran liegende Schicht beschränkt ist. Selektive Plattenexperimente zeigten, dass 50 Tage nach Animpfung des Reaktors der Anteil der aerob kultivierbaren Zellen, die fähig sind, Toluol zu metabolisieren, auf 40% gesunken ist. Eine beträchtliche Fraktion dieser Toluolabbauer – zwischen 40 und 70% – bestand aus Bakterien, die sich von den ursprünglich angeimpften Stämmen Pseudomonas putida F1 und Rhodococcus globerulus PWD1 unterscheiden.

Um Sensitivitätsanalysen und Massstabvergrösserung-Betrachtungen durchführen zu können, wurde ein Modell des neuen Prozesses entwickelt. Die vier Phasen – Gas, Absorbens, Biofilm und Kulturmedium – wurden unter Annahme des Gleichgewichts an ihren Grenzen modelliert. Für jede Phase wurden Differentialgleichungen für die Sauerstoff-
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# Nomenclature

## Latin characters

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<th>Symbol</th>
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<tr>
<td>( b )</td>
<td>m</td>
<td>Width</td>
</tr>
<tr>
<td>( c )</td>
<td>( g/m^3 )</td>
<td>Concentration</td>
</tr>
<tr>
<td>( D )</td>
<td>( m^2/s )</td>
<td>Diffusion coefficient</td>
</tr>
<tr>
<td>( h )</td>
<td>m</td>
<td>Height</td>
</tr>
<tr>
<td>( H_{ga} )</td>
<td>-</td>
<td>Partition coefficient between the gas-phase and the absorbent-phase ( \frac{c_g}{c_a} )</td>
</tr>
<tr>
<td>( H_{ma} )</td>
<td>-</td>
<td>Partition coefficient between the mineral-medium and the absorbent ( \frac{c_m}{c_a} )</td>
</tr>
<tr>
<td>( I )</td>
<td>-</td>
<td>Fluorescent intensity</td>
</tr>
<tr>
<td>( j )</td>
<td>( g/m^2s )</td>
<td>Flux</td>
</tr>
<tr>
<td>( k )</td>
<td>( m/s )</td>
<td>Mass transfer coefficient</td>
</tr>
<tr>
<td>( K_S )</td>
<td>( g/m^3 )</td>
<td>Half-saturation constant</td>
</tr>
<tr>
<td>( l )</td>
<td>m</td>
<td>Length</td>
</tr>
<tr>
<td>( \dot{m} )</td>
<td>( g/m^2s )</td>
<td>Mass flux</td>
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<td>( \dot{M} )</td>
<td>( g/s )</td>
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<tr>
<td>( M )</td>
<td>( g/mol )</td>
<td>Molar mass</td>
</tr>
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<td>( \dot{n} )</td>
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</tr>
<tr>
<td>( OTR )</td>
<td>( mg/lh )</td>
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</tr>
<tr>
<td>( p )</td>
<td>Pa</td>
<td>Pressure</td>
</tr>
<tr>
<td>( R )</td>
<td>( J/mol K )</td>
<td>Ideal gas constant = 8.314 ( J/mol K )</td>
</tr>
<tr>
<td>( SEC )</td>
<td>( g/m^2h )</td>
<td>Surface elimination capacity</td>
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XIV NOMENCLATURE

\( r \ \text{g/m}^3\text{s} \) \hspace{1cm} \text{Removal rate}

\( RE \ \% \) \hspace{1cm} \text{Removal efficiency}

\( T \ \text{K} \) \hspace{1cm} \text{Temperature}

\( t \ \text{s} \) \hspace{1cm} \text{Time}

\( \bar{v} \ \text{m/s} \) \hspace{1cm} \text{Superficial velocity}

\( V \ \text{m}^3 \) \hspace{1cm} \text{Volume}

\( \dot{V} \ \text{m}^3/\text{s} \) \hspace{1cm} \text{Volume flow rate}

\( x \ \text{g/m}^3 \) \hspace{1cm} \text{Concentration of biomass}

\( Y \ \) \hspace{1cm} \text{Yield}

\textbf{Greek characters}

\( \beta \ \text{m/h} \) \hspace{1cm} \text{Mass transfer coefficient}

\( \eta \ \text{Pa·s} \) \hspace{1cm} \text{Dynamic viscosity}

\( \mu \ \text{1/h} \) \hspace{1cm} \text{Specific growth rate}

\textbf{Superscripts and subscripts}

\( 0 \) \hspace{1cm} \text{Original, inlet, initial}

\( a \) \hspace{1cm} \text{Absorbent-phase}

\( \text{abs} \) \hspace{1cm} \text{Absorbed/absorber}

\( b \) \hspace{1cm} \text{Biofilm-phase}

\( \text{biof} \) \hspace{1cm} \text{Biofilm}

\( \text{bleed} \) \hspace{1cm} \text{Bleed stream}

\( \text{conv} \) \hspace{1cm} \text{Convective}

\( \text{diff} \) \hspace{1cm} \text{Diffusive}

\( \text{feed} \) \hspace{1cm} \text{Feed stream}

\( g \) \hspace{1cm} \text{Gas-phase}

\( \text{green} \) \hspace{1cm} \text{Green emitted fluorescence}

\( i,j \) \hspace{1cm} \text{Running parameters, substrate}
m Nutrient-medium-phase
max Maximum
O₂ Oxygen
ov Overall
S Substrate
T Toluene
tot Total
out Outlet, flowing out

Abbreviations

BOD Biochemical oxygen demand
CFD Computational fluid dynamics
CFLSM Confocal laser scanning microscopy
CFU Colony forming unit
CoA Coenzyme A
DAQ Data acquisition
DMSO Dimethylsulfoxide
dNTPs Deoxyribonucleotides
EDTA Ethylene diamine tetra acetic acid
EM Electron microscopy
EPA Environmental protection agency
EPS Exopolymeric substances
FID Flame ionization detector
FITC-ConA Fluorescein Isothiocyanate-Concanavalin A
GC Gas chromatography
HPLC High performance liquid chromatography
hpp Hydroxy phenyl propionate
LB Luria-Bertani medium
MM Minimal medium
NA Numerical aperture
NI National Instruments
OD₆₀₀ Optical density at a wavelength of 600 nm
OC  Steady state operating condition
ODE  Ordinary differential equation
P28  Membrane test cell P28 (CM-CELFA)
PBS  Phosphate buffer saline
PCR  Polymerase chain reaction
PDA  Photodiode analyzer
PDMS Poly dimethyl siloxane
PMT  Photo multiplier tube
PP  Polypropylene
PTFE Poly tetra fluor ethylene (Teflon)
RBC  Rotating biological contactor
ROI  Region of interest
SAFEL Swiss agency for environment, forest and landscape (BUWAL)
TAE  Tris acetic acid-EDTA
TOC  Total organic carbon
VOC  Volatile organic compound

**Dimensionless numbers**

\[ Nu \quad \text{Nusselt number} \]
\[ Re \quad \text{Reynolds number} \]
\[ Sc \quad \text{Schmidt number} \]
\[ Sh \quad \text{Sherwood number} \]
Chapter 1

Introduction to the treatment of volatile organic compounds

The emissions of volatile organic compounds (VOCs) are becoming an increasing regulatory concern. In 2001, in Switzerland alone, 70'000 t/a of anthropogenic VOCs were emitted into the environment (SAFEL, 2003). Most emitted VOCs contribute substantial damage on fauna and flora (Barnes, 1998) and are likely to be harmful to human health, generating nausea, headaches, irritation, affecting the nervous system and lungs (Delhomenie and Heitz, 2005). Another indirect problem, caused by some VOCs, is the formation of ozone in the troposphere by solar irradiation during summer time. A certain fraction of these emissions cannot be eliminated, simply because they originate from private households or open systems. 20% of emissions stemmed from private households, 80% from the industry; from which approximately 30% are caused by printing and paint manufacturing companies. These industries often use poorly water soluble solvents, and the waste gas streams are often subject to load fluctuations.

The maximum allowed mass of pollutants emitted into the atmosphere
is regulated through legislation. In Switzerland, the legal limit is defined in the Ordinance on Air Pollution Control (LRV) of 16 December 1985, revised March 2000 (SAFEL, 2005). The gaseous organic substances are subdivided into three classes, with the following limiting values:

a. Class 1 substances at a mass flow rate of 0.1 kg/h or more: 20 mg/m³
b. Class 2 substances at a mass flow rate of 2.0 kg/h or more: 100 mg/m³
c. Class 3 substances at a mass flow rate of 3.0 kg/h or more: 150 mg/m³

The different organic substances are classified according to their affects on human health or the environment in general.

1.1 Biological waste gas treatment

Biological degradation is an attractive option for the purification of emissions in a low concentration range (< 5 g/m³) as well as of mixtures of pollutants, which cannot be economically recycled. Due to its simplicity, environmental sustainability and cost-effectiveness, biological treatment is often a good alternative to conventional air pollutant control technologies, such as incineration, catalytic or UV/photochemical oxidation, adsorption, absorption, condensation or membrane processes (Delhomenie and Heitz, 2005; Ottengraf and Diks, 1992).

Biological degradation is achieved at ambient temperatures, it does not generate secondary problematic compounds such as nitrogen oxides or loaded adsorbents and is positively perceived by the general public (Mening and Krill, 1997). VOCs are converted into carbon dioxide by living microorganisms, suspended in an aqueous solution or immobilized in biofilms.

Currently used biological waste air treatment processes are biofilters, biotrickling filters, rotating biological contactors (RBC), membrane bioreactors and bioscrubbers (Rüdiger, 1999).

Biofilters are the most widely used bioreactors for air pollution control. Biofiltration technology is the oldest biotechnological method to remove
1.1. BIOLOGICAL WASTE GAS TREATMENT

undesired odorous compounds as well as low concentrations of VOC from off-gases. It has been in use since the 1920s (Van Groenestijn and Hesselink, 1993) and is well established today (Cox and Deshusses, 2002; Van Lith et al., 1997) for the treatment of a wide range of pollutants (e.g. Devinny et al., 1999; Kennes and Thalasso, 1998). The reasons for its popularity are the simple process engineering as well as the low capital and operating costs (Devinnny et al., 1999). Biofilters are reactors in which a polluted air stream is passed through a porous packed bed (generally a peat or compost mixture) on which a mixed culture of pollutant degrading organisms is naturally immobilized (Deshusses, 1997). Control of the humidity, pH and the supply of inorganic substances is difficult or impossible in biofilters, because of the mode of operation with no or little free water. However, control of the pH is required for the treatment of acid forming pollutants, such as chlorinated hydrocarbons, organo-sulphur compounds, hydrogen sulfide, and ammonia (Zuber et al., 1997), since low resulting pH would inhibit bacterial growth.

Therefore, biological treatment of acid forming compounds is often done in biotrickling filters (Zuber, 1995). A distinct free water phase containing various nutrients is trickled over an inert packed bed, consisting of random or structured packing materials, which are made of inert materials, such as plastic, lava rocks, polyurethane foam, etc. (Cox et al., 1998; Smith et al., 1998). However, long-term operation is often affected by biomass accumulation (Woertz et al., 2002), which rapidly leads to an increased pressure drop and finally to clogging of the reactor (Alonso et al., 1997; Weber and Hartmans, 1996).

A novel promising solution to avoid clogging is the rotating biological contactor (RBC) for waste gas treatment. This technique has been adopted from waste water treatment, where it is well established (Antonie, 1976). The biofilm grows on rotating disks partly submerged in a nutrient solution. The movement of the disks through the aqueous phase introduces shear stress on the biofilm, hindering an excessive accumulation of biomass, which would lead to clogging of the disks and the reactor (Rudolf von Rohr and Rüdiger, 2001). The waste air is introduced to the reactor through a hollow shaft, on which the disks are mounted (Vinage, 2002).

Suspended bacteria are used to degrade the VOC in bioscrubbers. This
type of reactor consists of an absorber and an aerated tank bioreactor similar to activated sludge processes. The scrubbing liquid in the absorber is the culture medium, which stems from the stirred bioreactor. The considerably large volume of the aqueous phase makes this system ideal for waste gas streams which are subject to fluctuating loads (Van Groenestijn and Hesselink, 1993). However, this buffering is only suitable for highly water soluble compounds. The cell density is lower for the suspended biomass reactors than for those working with biofilms.

Membrane bioreactors are particularly promising for the treatment of poorly water soluble compounds, which feature high air-water partition coefficients. This characteristic causes a low VOC concentration in the aqueous phase hampering the removal of the VOCs from the air through the aqueous phase by biological means. Membrane bioreactors are especially favorable for poorly water soluble compounds. In membrane bioreactors a large contact area between the two phases can easily compensate for the resulting small mass transfer rate from the gas to the water phase, wherein a membrane separates the gas from an aqueous phase. The organic pollutants diffuse through the membrane into the water phase, where they are readily degraded by microorganisms, which can grow as planktonic cells (Reiser et al., 1997) or they form biofilms on the membrane surface (Ergas et al., 1999; Parvatiyar et al., 1996; Reij et al., 1995).

1.2 Challenges to biological waste gas treatment

In spite of the large variety of proposed waste gas treatment systems and the extensive research carried out in this field, biological waste gas treatment processes face major challenges requiring extensive fundamental scientific research. In the following three sections these challenges are discussed based on published research studies.
1.2.1 Fluctuating pollutant loads and their buffering

Biological waste gas treatment systems are sensitive to transient feed conditions and process shut-downs (Al Rayes et al., 2001; Choi et al., 1998; Cox and Deshusses, 2002). Short-term inlet fluctuations of VOCs can reduce the performance of a bioreactor by stressing the microorganisms (Al Rayes et al., 2001). Periods of low concentration or process shutdowns that interrupt the flow of VOCs can harm the biology in such a way that the reactor needs a long time (several days to a week) to fully recover (Cox and Deshusses, 2002; Martin and Loehr, 1996). High peak concentrations can be toxic to the bacteria in the reactor and exceed its treatment capacity. In both cases waste gas leaves the reactor untreated and contaminates the environment (Weber and Hartmans, 1995). This result, along with the impact on the bioreactor can be reduced by buffering the VOCs prior to biological degradation, thereby removing high peaks and bridging periods of no VOCs in the gas. Different physical or chemical approaches such as absorption or adsorption were examined.

Absorption is performed by using an aqueous phase as a buffer for polar compounds in e.g. bioscrubbers (Van Groenestijn and Hesselmink, 1993). For apolar compounds the buffering effect of the aqueous nutrient solution can be improved by adding water-immiscible organic solvents, which are dispersed into the aqueous medium (Al Rayes et al., 2001; Cesario et al., 1997a; Collins and Daugulis, 1997). The hydrophobic pollutants are removed from the waste gas stream and buffered by absorption in the secondary organic phase. In the case of low inlet gas concentrations, the buffered VOCs can be stripped from the absorption medium and contaminate the off-gas. Therefore these absorption processes can be used to balance the fluctuating gas concentrations and to ensure the continuous delivery of VOCs to bioreactors.

Adsorption processes in combination with biological waste gas treatment systems are commonly carried out in one of three ways. The adsorbent is directly incorporated into the biofilter or it is placed in front or after it (Adami and Kümmel, 1997; Li and Moe, 2005; Ottengraf, 1986; Tang and Hwang, 1997; Weber and Hartmans, 1995). The contaminants from the waste gas are adsorbed at high concentrations and readily desorbed at low concentrations. In doing so, adsorbents remove VOCs from the gas phase or deliver them to the waste gas stream and dampen the
load fluctuations. In the case where the adsorbent is placed directly into the reactor the presence of bacterial populations on the surface of the adsorbent leads to the formation of a biofilm over the entire surface (Mason et al., 2000). This increases the resistance of the mass transfer of pollutants from the waste gas stream to the adsorbent. Furthermore, this buffering configuration is not able to dampen high peaks before the waste gas comes in contact with the microorganisms. These high peaks can be toxic to bacteria and therefore have to be avoided.

1.2.2 Poorly water-soluble compounds

Poorly water soluble compounds feature high air-water partition coefficients, causing low water concentrations and thereby hampering the removal from waste gas (Brindle and Stephenson, 1996). There are basically two methods to biologically treat poorly water soluble VOCs, either by using (extractive) membrane bioreactors (Jorge and Livingston, 2000), or by using organic liquids to improve the elimination of less soluble compounds (Cesario et al., 1997b; Collins and Dauguilis, 1997, 1999).

Extractive membrane bioreactors have been developed from pervaporation processes by exchanging the vacuum phase with a phase of culture medium, where biodegradation mechanisms maintain the concentration gradient needed to transfer organic pollutants across the membrane (Nguyen and Nobe, 1987). The waste gas passes on one side of the membrane, the VOCs diffuse through the membrane and are readily degraded by the bacteria present in the culture medium. This membrane process is favorable for poorly water soluble compounds, because the pollutants do not have to be absorbed directly into the aqueous medium to be made accessible for the microorganisms, and because the user-defined high membrane area (Brindle and Stephenson, 1996) can compensate for the unfavorable partition coefficients of the VOCs. The composition of the nutrient medium does not affect the waste gas separated by a membrane, therefore the condition within the bioreactor can be optimized for high biodegradation rates (Livingston, 1993a,b). Other advantages of membrane biofilters for the treatment of VOCs are the prevention of gas stripping from the aqueous medium, the
compactness of the system and the independent control of the waste
gas stream and nutrient flow rate (Parvatiyar et al., 1996). However,
excessive biofilm growth is one of the major drawbacks of membrane
biofilters. The accumulation of biomass can lead to membrane fouling,
resulting in mass transfer limitation of substrates (VOCs and oxygen)
leading to a decline of biomass activity and finally to the breakdown of
the reactor.
Generally, two kinds of membranes are used, dense polymeric mem-
branes, where the material selectively permeates the solutes, and porous
non-selective membranes, which act simply as barriers and support for
the active organisms. Work done with dense polymer membranes mainly
focused on silicone membranes, which have a high oxygen permeability
and are very resistant to chemical and mechanical abrasion (Brindle
and Stephenson, 1996). Nevertheless, a higher mass transfer resistance
than for microporous hydrophobic membranes is suspected. In dense
membranes the contaminants have to dissolve into the membrane ma-
terial and diffuse through it, whereas in porous membranes, diffusion
takes place in air-filled pores (Reij et al., 1995).
The addition of an organic phase to the nutrient medium is another
method to increase the rate of transport towards the water phase.
The dispersion of a water-immiscible organic solvent into the aqueous
medium, in which these apolar compounds are preferentially soluble,
improves transport (Daugulis, 2001; Rols et al., 1990). Examples of
these organic liquids include hydrocarbons and perfluorocarbons (Ju et
al., 1991). Although the overall solubility of VOC and oxygen in the
dispersion can be increased considerably, the efficiency is dependent on
the transfer of the apolar compounds between the organic phase and
the water phase. This mass transfer rate is directly proportional to the
interfacial area between the two liquids (Cesario et al., 1997b). The
emulsifying of the two phases is a highly energy consuming process, and
thorough mixing, as well as separation, are often difficult to achieve
(Kollmer, 1997).
1.2.3 Biomass accumulation in fixed film bioreactors

Clogging of fixed film bioreactors caused by excessive biomass growth is one of the main obstacles to the industrial application of biological waste gas treatment (Cox and Deshusses, 1999b; Sorial et al., 1995; Weber and Hartmans, 1996). Due to rapid biomass accumulation in the packed bed, the pressure drop increases. At the same time, pollutant removal declines mostly because of the decrease in interfacial area for mass transfer (Alonso et al., 1997). This accumulation is a complex phenomenon determined by such diverse factors as the biological and physical characteristics of the compounds involved, their microbial degradation rates, the mechanical and morphological characteristics of the formed biofilm and the structural properties of the packing material (Okkerse et al., 1999). To extend the life time of bioreactors, several strategies have been proposed to either prevent the reactors from accumulating biomass, or to remove it. These refer to mechanical, chemical and biological methods.

Biological means include a reduction of the microbial growth rate by nutrient (Allan et al., 2002; Weber and Hartmans, 1996; Wubker et al., 1997) or moisture limitation (Garcia-Pena et al., 2001), the addition of growth-inhibiting concentrations of NaCl in the recycle liquid (Schonduve et al., 1996) and periodic starvation of the microorganisms (Cox and Deshusses, 2002). For the latter, intermittent treatment of the waste gas would require at least two reactors to ensure continuous operation, which would be reflected in high investment costs. The use of protozoa, mites and fly larvae that prey upon biomass have been studied for controlling biomass accumulation (Cox and Deshusses, 1999b; Prado et al., 2002; Won et al., 2002). Another method to prevent excessive growth of biomass is the replacement of ammonium with nitrate as a nitrogen source (Jorio et al., 1998), wherein more energy is used for bacterial growth, leading to a reduced biomass yield (MacFarlane and Bagley, 1997).

Chemical methods include washing the packed bed with aqueous solutions of chemical substances to remove excessive biomass (Cox and Deshusses, 1999a; Weber and Hartmans, 1996). Chemicals tested for such procedures are solutions of single substances or mixtures of e.g. NaOH, NaClO or H₂O₂.
1.3. OBJECTIVES

Mechanical removal techniques of accumulated biomass include periodic backwashing of the reactor with water (Smith et al., 1998; Sorial et al., 1995), periodic stirring of the randomly packed bed (Laurenzis et al., 1998; Wubker et al., 1997), as well as directionally switching operations (Song and Kinney, 2000). A different approach is to introduce the Rotating Biological Contactor (RBC) principle, where the continuous rotation of the carrier applies shear stresses on the biofilm to prevent clogging and maintenance shutdown (Vinage, 2002).

Unfortunately, there are various drawbacks to these methods. Limiting bacterial growth decreases bacterial activity, and hence, also decreases the pollutant elimination capacity. Chemical biomass removal results in partial or complete inhibition of microbial activity for a considerable period and affects the production of waste water. Mechanical methods require large complex facilities, e.g., for bed expansion during fluidization in case of random packing media (Sorial et al., 1995). Scale-up of these systems might be complicated or even impossible and they generate large amounts of waste water (Cox and Deshusses, 1999b).

An improved, biological waste gas treatment process is desired, which is able to cope with:

- VOC load fluctuations in the waste gas stream,
- hydrophobic VOCs and
- excessive biomass accumulation.

1.3 Objectives

The aim of this present work is to develop and characterize a novel waste gas treatment system. One that is designed to buffer fluctuating VOC loads as well as oxygen, degrade poorly water soluble VOCs and prevent biomass accumulation,
which might lead to clogging of the reactor. The system shall not ex-
hibit aging effects during the long-time experiment and shall only require
minimal maintenance.

The characterization of the system is based on experimental investiga-
tions as well as on modeling of the facility. The investigations include
analyses of the degradation performance under steady-state conditions
as well as of the buffering capacity of the reactor under dynamic condi-
tions. The biofilm is analyzed for its surface profile and its thickness, its
activity profile along the thickness of the film and its bacterial composi-
tion. The modeling of the system increases the fundamental theoretical
understanding of the process and provides the methodology for reactor
design and scaling-up studies.
Chapter 2

Design of the experimental waste gas treatment facility

The system presented here overcomes the current drawbacks and challenges in biofiltration. The system allows:

- treatment of fluctuating loads,
- buffering of VOCs,
- treatment of compounds with low water solubility and
- continuous removal of excess biomass to prevent the reactor from clogging.

These challenges are met by introducing a novel, two-step membrane bioreactor. In the first step the VOCs are withdrawn from the waste gas by absorption into silicone oil. In the second step the VOCs are desorbed from the absorbent by biological degradation in a membrane
biofilm reactor (see Figure 2.1).
In the following sections the concept and its realization are explained in detail.

2.1 Concept of the novel waste gas treatment system

Figure 2.1: The concept of the novel waste gas treatment system includes a buffering of the VOCs and oxygen, prior to biological degradation of these substrates. The inactive biomass, the top layer of the biofilm, is eroded by the flow of nutrient medium over the biofilm, preventing the reactor from clogging.

Figure 2.1 shows a schematic of the two-step membrane bioreactor. The VOCs are removed from the contaminated waste gas by membrane based absorption. The VOCs, together with oxygen from the waste gas, diffuse through a first membrane that separates the gas from the absorbent, and are readily buffered in the absorbent. This buffering is introduced to remove the VOCs from the waste gas independent of load fluctuations.
and microbial performance. The total volume of the absorbent is well mixed; therefore, the VOCs and the oxygen are transported by convection in the absorbent. In the second process step the VOCs are degraded by bacteria, while a second membrane separates the absorbent from the mineral medium. An aerobic biofilm grows on the mineral medium side of this membrane and degrades the VOCs buffered in the absorbent. In this membrane bioreactor, the mineral medium flows along the biofilm, on the one hand drenching it with nutrients and trace elements necessary for bacterial growth, on the other hand introducing shear stress on the surface and thereby removing excess biomass to prevent the reactor from clogging.

The VOCs reach the biofilm by diffusion through the second membrane. The necessary oxygen for the aerobic, microbial degradation also stems from the absorbent. Oxygen is buffered along with the VOCs. This feed of the VOCs and oxygen to the base of the biofilm creates an inverse biofilm, with the base layer, close to the membrane, as the most active zone. This activity distribution is different from natural biofilms, where the most active layer is usually in the top zone next to the aqueous medium, which delivers the substrates. This inverse activity profile is advantageous. The flow of the medium introduces shear stresses on the biofilm surface and thereby removes the top layer of the biofilm by erosion or sloughing. Due to the inverse activity profile, inactive biomass is discharged from the reactor. The active base layer is not harmed during this process and the microbial activity remains high at all times.

2.2 Design and Mode of Operation

The process itself and the experimental facility are described in the following subsections.

The set-up is built using flat membranes for the absorption as well as for the bioreactor unit. The use of membranes has the following advantages compared to directly contacting the distinct phases:

- The feed of VOCs and oxygen to the base layer creates an inverse biofilm, featuring the most active zone at its base.
• A membrane constitutes an exact phase separation. There is no risk of cross-contamination between the two separated fluids. During absorption, dust or other particulate matter present in the waste-gas stream cannot contaminate the absorbent; at the same time the absorbent cannot contaminate the gas by droplet entrainment. In the case of the bioreactor, the membrane separates the absorbent from the nutrient medium. The absorbent is therefore not contaminated with biomass and the medium is not contaminated with absorbent.

• The flow profile along a flat surface in a channel is known. This allows estimation of the influence of the shear stresses on the biofilm erosion.

• The use of flat membranes does not impose any restrictions on the choice of the membrane material because of geometrical constraints. The membrane material is important because of its chemical properties (selectivity, resistance to solvents) and its construction, since the biofilm grows on the membrane (adhesion, fouling).

Additional experiments, besides the investigation of the degradation performance of the reactor, require direct access to the biofilm to take samples during reactor operation and to acquire dissolved oxygen concentration profiles perpendicular to the biofilm surface. The biofilm sampling as well as the oxygen profiling demand that the level of the culture medium flowing along the biofilm does not exceed a few centimeters above the biofilm surface. Manual biofilm sampling on a fragile membrane is difficult without breaking it; when the biofilm is deeply submerged, sampling is virtually impossible. The oxygen microsensor, measuring only 10 cm in length, is mounted on a linear track guide, which is not allowed to be submerged. Therefore, the level of the medium above the biofilm must not exceed few centimeters at one position in the reactor during operation.
2.2.1 Flow chart

The described process is realized in an experimental facility by locally separating the absorption and the biological degradation step (see Figure 2.2). This is mainly done to allow planning and optimization of each unit operation separately. The waste-gas stream and the absorbent are brought in contact in the absorption-module. A membrane separates the gas from the absorbent phase. The absorbent is carried in a closed, virtually ideally mixed loop, which connects the absorption-module with the bio-module, where the desorption of the buffered substances takes place. In this module, a membrane separates the absorbent from an aqueous phase, the growth environment for the microorganisms. Bacteria grow immobilized in a biofilm and are overflown by the nutrient phase. The nutrient solution is carried in a loop, where a feed stream carries fresh medium to the reactor, while a bleed stream discharges culture liquid at the same rate.
2.2.2 Flat membrane modules

The design of the absorption-module and the bio-module is identical, where a flat membrane separates two channels from each other. In the absorption-module, the membrane separates the gas from the absorbent. Compared to the conceptual drawing (see Figure 2.1), the realization is upside down, where the absorbent flows in the lower channel, and the gas is above the membrane (see Figure 2.3). The absorbent
is streamed in the lower compartment, because in case of operation failure requiring shut down of the pumps, the absorbent would stay in the lower channel and not create buoyancy of the membrane. In the bio-module the membrane separates the absorbent from the aqueous phase. The absorbent flows again in the lower channel, while the nutrient medium flows in the upper channel. On the nutrient medium side of the membrane a biofilm develops (see Figure 2.4).

Figure 2.5 shows exemplarily an exploded view of a membrane bio-module. The module is built of a base plate and an upper plate. In the upper plate an additional hole is cut out for fitting a glass plate enabling viewing of the membrane. Two pockets are countersunk into both plates. The membrane is clamped in between the two plates, separating the pockets from each other and thereby forming two channels.

**Figure 2.5:** Photo-realistic, exploded view of a membrane module.
The nutrient medium flows through the upper channel, the absorbent through the lower one. The nutrient medium is conducted in a pipe from the tank to the module. The pipe is connected to a distribution component, which is fixed on top of the upper plate. It holds a narrow channel, which distributes the liquid along the width of the module. Bores in the plate lead the nutrient medium to the pocket in the upper plate. These bores have to be appropriately designed to introduce an additional pressure drop, forcing the fluid to be distributed evenly along the width of the module. This is easily achieved, if the sum of the cross-sectional areas of the bores is smaller than the cross-sectional area of the supply pipe. The nutrient medium leaves the module, analogously to the inlet system, through bores and a collecting component.

All seals in the membrane module are fitted with O-rings. The O-ring nuts are countersunk into the ground plate. The inner O-ring seals the channels from each other while the outer one prevents the fluid from leaving the reactor (see Figure 2.6).
2.2. **DESIGN AND MODE OF OPERATION**

### 2.2.3 Materials and Dimensions

All components of the membrane modules are made of stainless steel (V4A) and glass. The O-rings used to seal the membranes are made of Viton (fluoroelastomer). The glass planes of the bio-modules are glued into the steel cover plates by a special adhesive used in the airplane industry to seal fuel tanks (PR1422, Hutchinson, Langnau a/A, Switzerland). All these materials are chemically resistant against toluene and oxygen and they do not negatively influence bacterial growth.

The membrane areas for the absorption-module and for the bio-module amount to 0.181 m² and 0.275 m², respectively. The channel heights, volume flows and resulting superficial velocities and residence times are shown in Table 2.1.

<table>
<thead>
<tr>
<th></th>
<th>absorption-module</th>
<th>bio-module</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gas absorbent</td>
<td>absorbent mineral medium</td>
</tr>
<tr>
<td>h mm</td>
<td>5</td>
<td>10.7</td>
</tr>
<tr>
<td>V l/min</td>
<td>1 - 4</td>
<td>0.35</td>
</tr>
<tr>
<td>( \dot{\bar{v}} ) m/s</td>
<td>1.25 ( \times ) ( 10^{-2} )</td>
<td>2 ( \times ) ( 10^{-3} )</td>
</tr>
<tr>
<td>( \tau ) s</td>
<td>13.7 - 54.7</td>
<td>250</td>
</tr>
</tbody>
</table>

A range of the gas volume flow is stated, due to the different experimental conditions (see Table 3.1). The superficial velocity and the residence time of the absorbent in the absorption module are not known, since the total volume flow rate of the circulated liquid is not measured. Only the flow rate of absorbent through the bio-module is measured. The total volumes of absorbent and mineral medium circulated in the experimental facility amount to 7 l and 10 l, respectively.
2.2.4 Membrane support

The following challenges arise during the design of the flat membrane modules: how to keep the membranes stable in place, and how to mix the absorbent in the module virtually ideally, without increasing the risk of membrane breakage. In filtration or pervaporation processes the membrane is usually pressed against a sinter metal, which serves as a support structure. The analogous procedure of a transmembrane pressure difference is applied in our case, but with a different support material. Sinter metals are not suitable as a support structure for our facility. The absorbent features a considerably higher viscosity than water, which would lead to a very high pressure drop over the module, requiring an even higher pressure on the membrane side turned away from the absorbent.

The main goal of a mixing process is to prevent the formation of thick, quiescent fluid boundary layers on the membrane, which would increase the mass transfer resistance across the membrane. The simplest way to reduce the boundary layer thickness is to form a turbulent fluid regime. However, this is not possible, since the viscosity of the absorbent would generate a high pressure drop, making it impossible to maintain a small transmembrane pressure difference to hold the membrane on the support structure, as compared to the high pressure drop along the module. Therefore, static mixers are used to overcome these difficulties and to form the support surface for the membrane (see Figure 2.7). The Sulzer QUADRO® (Sulzer Chemtech, Winterthur, Switzerland) mixers feature a quadratic cross-section. They are symmetrically built, repeating the cube-shaped mixing element (see Figure 2.8), which divides the fluid stream into four parts. The static mixers are placed next to each other in the pocket of the base plate to form a coherent support structure using their side edges and webs (see Figure 2.7). The mixers are originally designed to mix highly viscous two component resins. This feature is advantageous in the present case, because the absorbent is already mixed at low fluid velocities, creating a small, acceptable pressure drop along the module.

Static mixers are designed to mix two fluids in the direction of the flow, leading to a virtually complete mixture at the end of the mixing element. However, in our case, the sides of the static mixers are used as support
2.2. DESIGN AND MODE OF OPERATION

Figure 2.7: The base plate of the bio-module is partly filled with Sulzer QUADRO\textsuperscript{®} static mixers. The webs and edges of these mixers, placed besides each other, serve as support structure for the membrane. (The spiral lines on the bottom of the pocket are mirages of the metal resulting from the machining.)

surface for the membrane. Therefore, it is of great interest how the velocity profile along this surface develops. A model based on computational fluid dynamics (CFD), calculated with a commercially available code, CFX-5 (ANSYS, Otterfing, Germany), based on the finite-volume method – the model is not described in more details here – shows that there are stagnant regions in the corners of the mixing elements (see Figure 2.8). These stagnant regions are characterized by increased thicknesses of the fluid boundary layers and consequently by increased mass transfer resistances. Nevertheless, it can be concluded that the use of static mixers is a good solution for mixing the absorbent at low fluid velocities while at the same time supporting the membrane.

The single static mixing elements are closed on all four sides. This creates distinct lines of static mixers, between which the absorbent cannot be
Figure 2.8: The velocity profile is shown for two mixing elements of a static mixer. The superficial fluid velocity in the static mixer is $1 \cdot 10^{-3} \text{m/s}$. The flow direction is from the left to the right.

exchanged. Therefore, the absorbent flow has to be distributed over the width of the module prior to entering the static mixers. The absorbent is fed from the rear side of the module through above described bores to the pocket in the base plate. An elbow is screwed into this pocket (see Figure 2.5) to form a channel, which is closed by the overlapping upper plate of the membrane module (see Figure 2.6). The elbow has bores through which the absorbent must leave the channel to feed the distinct static mixer lines (the bores in the elbow are visible in Figure 2.7).

2.2.5 Course of pressure in the reactor

The different flow rates and the different fluid properties of the absorbent and the nutrient medium, as well as the static mixers in the absorbent channels, result in different pressure drops necessary for the flow of the absorbent or the aqueous phase. It is considerably higher in the case of
the absorbent. The higher absolute pressure in the upper channel of the module assures the bearing of the membrane on the support structure. The pressure difference between the upper and the lower channel, as a function of the position in the module, is schematically presented for a module of 1 m length, where the membrane separates the absorbent from the water (Figure 2.9). At the module inlet, the pressure difference be-

![Figure 2.9](image)

**Figure 2.9:** The course of the calculated pressure difference in relation to the ambient pressure for the absorbent and the water are shown. The pressure on the water side of the membrane is at all positions higher than the pressure of the absorbent, flowing through the static mixers.

tween the water and the absorbent is 300 Pa. This pressure difference is sufficiently high to keep the membrane in place. The pressure difference increases linearly with increasing distance from the module inlet. This difference is even more pronounced for the gas/absorption membrane, since the pressure drop of the gas over the module is smaller than the one of the water, although it is also for the water only in the range of 25 Pa. The resulting maximum pressure difference at the module outlet does not cause membrane breakage. Therefore, the absorption module is built according to the concept presented in figure 2.9. However, the increasing pressure difference along the membrane is not feasible in the case of the bio-module. This is due to two reasons, the biofilm investigation
procedures cannot be accomplished through a standing water column of up to 0.3 m (see introduction to Section 2.2) and unequal pressures, at different locations in the module, may lead to a non-uniform biofilm growth. Therefore, the bio-module is separated into four sub-units to reduce the maximum pressure difference at the reactor outlet. Between the modules additional pressure drops in the aqueous phase are introduced by installing valves. The resulting course of pressure within these modules is presented in figure 2.10. The course as well as the absolute values of

\[ \text{Figure 2.10: The pressure conditions are shown for the four subunits of the bio-module. One half of the modules are run in the overpressure, the other in the depression, compared to atmospheric pressure. The course of pressure allows to carry out the biofilm analyses in module 3.} \]

the transmembrane pressure difference is identical for all four modules. The pressure difference between water and absorbent increases from the inlet to the outlet in each module by 600 Pa. However, this subdivision alone, does not allow the realization of the biofilm analysis procedures. During operation one position of the reactor is required to present ambient pressure, enabling the opening of the reactor without interference during the flow of the culture liquid and easy access to the biofilm. Therefore, part of the reactor is run in depres-
2.2. DESIGN AND MODE OF OPERATION

sion compared to the atmospheric pressure. Regarding the absorbent, modules one and two are in overpressure \((p > \text{atmospheric pressure})\) while three and four are in depression. For the modules one to three the culture liquid is in overpressure and only in module four in depression. In module three, the level of the culture liquid reaches only 3 cm, which allows opening of the reactor to carry out biofilm analyses.

In the gas channel of the absorption module, an overpressure, compared to the maximum pressure in the absorbent channel (analogous to figure 2.9), is created by a dip pipe in a standing water column, which is open to the environment. The off-gas leaves the reactor via this pipe; by adjusting the immersion depth of the pipe, the backpressure can be easily regulated. The backpressure can only be determined empirically, because neither the exact flow rate of absorbent through the absorption-module nor the pressure drop, introduced by the static mixers, are known exactly.

In the bio-modules, the necessary overpressure on the culture medium side of the membrane is achieved by a higher primary pressure of the medium compared to the absorbent. The culture medium loop, as well as the absorbent loop comprise two tanks each. For both loops, there is one tank located below the bio-modules while the other is located above (see the instrumentation scheme in figure 2.12). The constant primary pressures of the absorbent and the culture medium are guaranteed by the fixed fluid levels in the upper tanks. The levels are kept constant by the installed overflows, which lead a branch current of the circulated fluid streams back to the lower tanks. These fluid streams, which bypass the bio-modules, make sure that the total respective fluid volumes are well mixed. The other branch current also flows through the four bio-modules back to the lower tank. The flow rates of these streams are measured by means of two rotameters installed behind the bio-modules. This flow pattern – streaming from the upper tanks, through the modules, to the lower tanks – causes the fluids to be pressed through the first two modules and to be sucked through the last two. To adjust the pressure ratios in all four bio-modules to the same conditions, according to figure 2.10, the pressure of the nutrient medium must be decreased between the modules, achieved by valves. The primary pressures can also, if required, be reduced by the installed valves between the upper
tanks and the first bio-module. The reference is the pressure of the absorbent between modules two and three, which has to be equal to the ambient pressure. The small introduced pressure drops are measured by standing fluid columns, connected to the pipes before and after the valve, respectively. The glass tubes, in which the level of the standing fluid is measured with a ruler, must be open to the atmosphere (see figure 2.11). A constant fraction of the culture medium has to be replaced,

Figure 2.11: The additional pressure drop between two bio-modules is introduced by a valve. The small pressure difference is measured by the height of standing water columns, which are open to the atmosphere.

therefore, fresh nutrient medium is fed to the culture medium loop. Culture medium is disposed from the loop at the same rate as the feed via an overflow, installed in the lower tank. The medium is withdrawn from the bottom of the tank and the minimum level in the tank is sustained by a superelevation of the off-flow pipe. Thus, the off-flow pipe is protected from clogging, as it is often encountered, if a standing tube is used as the overflow.
Figure 2.12: The process diagram of the experimental facility according to DIN 28004 with the main information on the instrumentation.
2.3 Secondary components

2.3.1 Loading unit

The artificial waste gas, consisting of ambient air, enriched with defined amounts of toluene, is produced by means of a loading unit. It consists of a liquid mass flow controller, a gas volume flow controller and an evaporation unit (all parts delivered by Bronkhorst, Reinach, Switzerland). The liquid flow rate is controlled by a coriolis mass flow controller (L0-FAC-00-0). The adjustable toluene flow rate can be varied in the range of 0.1 and 3 \( \frac{\text{g}}{\text{h}} \). The accuracy stated by the manufacturer is ±0.03 \( \frac{\text{g}}{\text{h}} \). The gas flow rate can be varied between 1 and 10 \( \frac{\text{L}}{\text{min}} \), with an accuracy of ±0.1 \( \frac{\text{L}}{\text{min}} \) (F-201C-FAC-33-V). The temperature of the evaporator (W-202-310-Z) is set to 125 °C.

2.3.2 Control of the liquid level

The liquid levels in the lower tanks, holding the absorbent and nutrient medium, are controlled using ultrasound distance sensors (UP 500, SNT Sensortechnik AG, Rümlang, Switzerland). The system uses an electromechanical control, which shuts down the pumps in case of too low or too high liquid levels.

2.3.3 Pumps

Two centrifugal pumps are used to circulate the absorbent and the nutrient medium in the respective loops. The pumps are equipped with a magnetic clutch. The pumps are AM-45-GF-A-V-R1-B-E-N-1 (45 W, 50 Hz, IP 21, Lutz) and AM-250-F-A-V-R2-B-E-N-1 (180 W, 50 Hz, IP 55, Lutz, RMS Fluid-Fördertechnik, Niederdorf, Switzerland) for the absorbent and the nutrient medium, respectively.
2.3.4 Piping

The piping of the absorbent and the nutrient medium loop are made in stainless steel (V4A) tubing. The absorbent and the nutrient medium loop are made of 3/8” and 3/4” tubing. These rather large diameters (in comparison to the membrane areas) increase the total liquid volumes, thereby increasing the set-up slackness, however they are necessary to minimize the pressure drops of the fluids streaming through the pipes.

2.3.5 Storage tanks

Four tanks are used in the present set-up, two for the absorbent and the nutrient medium respectively. To be able to see the contents of the tanks as well as to enable the connection of the steel tubing, a stainless steel and glass combination with teflon sealing is chosen. A wide glass tube is sealed by a flat, round steel base and covering plates screwed to the tube. This enables welding of the pipes to the ground plates of all tanks. The cover plates of the upper tanks hold the probes (pH, O₂, CO₂, temperature) and the cover plates of the lower tanks each hold a filling nozzle as well as the ultrasound distance sensors (see Section 2.3.2) to regulate the control levels and enable automatic emergency shut downs. The dimensions of the tanks are shown in Table 2.2.

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**Table 2.2:** The heights (h) and the inner diameters (i.d.) are listed for the four tanks installed at the experimental facility.

<table>
<thead>
<tr>
<th></th>
<th>absorption loop</th>
<th>nutrient medium loop</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>upper tank</td>
<td>lower tank</td>
</tr>
<tr>
<td>h</td>
<td>mm</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>i.d.</td>
<td>mm</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>150</td>
<td></td>
</tr>
</tbody>
</table>
2.3.6 Rotameters

The volume flows of absorbent and nutrient medium through the biomodules is measured using rotameters. In the absorbent loop a rotameter, especially calibrated for the used silicone oil, applicable for volume flows up to 1.6 l/min is installed (V200L27 1/2", Vögtlin Instruments, Aesch, Switzerland). The flow rate of mineral medium through the biomodules is measured using a rotameter applicable up to 1'000 l/min (V250 3/4", Vögtlin, Instruments, Aesch, Switzerland).
Chapter 3

Materials and methods

3.1 Operating conditions

The reactor is operated in a non-sterile configuration. None of the inlet or outlet flows are sterile filtered and the reactor can be opened during operation. This operational mode is chosen for two reasons: to build and operate the set-up more easily, and to follow the development of a bacterial consortium in a toluene degrading bioreactor.

The operating conditions of the reactor are characterized by the gas flow rate and the toluene mass flow rate. The facility is run under different steady-state conditions as well as under dynamic conditions. The steady-state conditions are used to investigate the performance of the bioreactor, while the dynamic conditions are used to analyze the buffering capacity.

3.1.1 Steady-state conditions

Nine different steady-state points are run during the operation of the reactor. They differ from each other by the inlet mass flow rate of toluene $\dot{M}_{in}^T$ and the gas flow rate $\dot{V}_{in}^g$. The operating points are summarized in
Table 3.1: The steady-state operating conditions under which the reactor is run. They are defined by the mass flow rate of toluene and by the gas flow rate into the reactor.

<table>
<thead>
<tr>
<th>$M_{\text{in}}^T$ (g/h)</th>
<th>0.6</th>
<th>0.6</th>
<th>0.6</th>
<th>0.3</th>
<th>0.3</th>
<th>0.3</th>
<th>0.15</th>
<th>0.15</th>
<th>0.15</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{in}}^g$ (l/min)</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>conc. ($g/m^3$)</td>
<td>10</td>
<td>5</td>
<td>2.5</td>
<td>5</td>
<td>2.5</td>
<td>1.25</td>
<td>2.5</td>
<td>1.25</td>
<td>0.625</td>
</tr>
<tr>
<td>number</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 3.1: Three different mass flow rates of toluene are combined with three different gas flow rates. These combinations give five different inlet concentrations. A concentration of 2.5 $g/m^3$ is adjusted for each gas flow rate, while the concentrations of 5 and 1.25 $g/m^3$ are set for two gas flow rates, and the highest and the lowest concentration are only set for one gas flow. This procedure helps to find out whether the absorption step is appropriately designed or not.

Biological waste gas treatment is commonly accepted to be used for VOC concentrations below 5 $g/m^3$. However, due to waste gas fluctuations there are always periods of higher inlet concentrations. Therefore one inlet concentration is set to 10 $g/m^3$. On the other hand, in industry periods of very low concentrations also occur, which explains the last operating point, featuring a toluene concentration of 0.625 $g/m^3$.

### 3.1.2 Dynamic conditions

Starting from a distinct steady-state working point experiments are carried out under dynamic conditions. The gas flow rate is kept at a constant value, while the mass flow rate of toluene is varied in short intervals. This simulates a possible working day at an industrial plant, in which the flow is generally subjected to load fluctuations. The toluene inlet gas concentrations in the experiments vary from 0 to 20 $g/m^3$. The aim of this dynamic operation mode is the analysis of the buffering capacity of the novel set-up. These dynamic conditions are investigated for two
gas flow rates, 1 and 2/\text{min}. The starting concentration of toluene in the waste gas is 5 g/m³ in both cases. Therefore, the dynamic investigations begin from the steady-state operating conditions two and four, respectively (see Table 3.1).

3.2 Waste gas

3.2.1 Model VOC

Toluene is chosen as the model VOC for this project. Toluene is widely used in the chemical industry and, together with benzene, ethylbenzene and xylene, among the most often cited VOCs contaminating the environment (Attaway et al., 2001). It is listed in the top priority lists for hazardous substances of e.g. the U.S. Environmental Protection Agency (EPA) or the Swiss Agency for Environment, Forest and Landscape (SAFEL). Therefore, a large number of scientific studies in the area of waste gas treatment are carried out using toluene as the model VOC. This allows comparison of the obtained results with literature data while at the same time investigating an environmentally important VOC.

3.2.2 Analysis methods

The inlet and outlet gas flows of the reactor are analyzed for concentrations of toluene, CO₂ and temperature. Only the measuring methods for the leaving stream are describe below. The toluene concentrations of the inlet flow is taken on from the loading unit. The oxygen content in air is assumed to be constant at 20%.

3.2.2.1 Toluene

The toluene concentration in the gas at the outlet of the reactor is measured using a flame ionization detector (FID) (VE7, J.U.M. Engineering,
Based on the assumption that toluene is the only organic carbon source in the gas, this analysis can be used to calculate the toluene gas concentration at the outlet of the equipment. This is a reasonable assumption since the concentration of VOCs in the troposphere is normally negligible, compared to the measured toluene values, which are in the range of 50 to 2000 ppm. Furthermore, it is unlikely that the bacteria produce intermediate organic-gaseous degradation products.

The available FID has an accuracy of ±1.5 % of the calibration point. It is calibrated with nitrogen (quality 5.0) to set the zero-point. The sensitivity calibration for the steady state working points one to five is done using a calibration gas made of 8066 ppm propane in nitrogen (quality 5.0), while for the working points six to nine the calibration gas is made of 763 ppm propane in nitrogen (quality 5.0) (PanGas, Dagmersellen, Switzerland). The toluene concentration, expressed in the unit ppm, is calculated according to the ratio of the number of C-atoms in a toluene and in a propane molecule. The response factor between toluene and propane, analyzed with the FID VE7, is 1.02 (Redmann, 1990).

The toluene concentration ppm ($c_T^{\text{ppm}}$) is calculated from the given concentration in g/m$^3$ ($c_T^{\text{g/m}^3}$) according to the ideal gas law as described by Equation 3.1.

$$
c_T^{\text{ppm}} = \frac{n_T}{n_{g,\text{tot}}} 10^6 = \frac{M_T}{M_{g}} \frac{RT}{n_T} 10^6 = c_T^{\text{g/m}^3} \frac{RT}{M_T p} 10^6
$$

where $n$ denotes the molar flow rate, $M_T$ the molar mass of toluene, $R$ the ideal gas constant, $T$ the temperature and $p$ the pressure.

### 3.2.2.2 CO$\textsubscript{2}$

The carbon dioxide concentration (CO$_2$) in the gas is analyzed with an infrared gas analysator (UNOR610, Maihak, MBE Electronic AG, Schwerzenbach, Switzerland). The calibration is done with nitrogen to set the zero-point, and 8.01 % of CO$_2$ in nitrogen (quality 5.0) (PanGas, Dagmersellen, Switzerland).
3.3. ABSORBENT

3.2.2.3 Temperature

The temperatures of the in-flowing and out-flowing gas streams are measured using type K thermocouples (Thermocoax, Thermocontrol Dietikon, Switzerland), measuring from 233 K to 1273 K with an accuracy of ±1.5 K.

3.3 Absorbent

3.3.1 Selection

The absorbent is selected based on its partition coefficients for oxygen and VOCs with a low water solubility. The gas-absorbent partition coefficients, defined as the gas concentration divided by the absorbent concentration, should be minimized. Small partition coefficients force the two substrates in question to change phase from gas to absorbent, which means that VOCs with a low water solubility are removed from the waste gas by absorption.

The desorption of the VOCs and the oxygen from the absorbent is carried out by biological degradation. These absorbed substrates have to undergo a phase transfer from the absorbent to the culture medium to be available for the bacteria. In order not to additionally limit the bacterial degradation by mass transfer of the substrates from the absorbent to the aqueous phase, the partition coefficients between the absorbent and the aqueous phase should not be too small. This coefficient is herein defined as the concentration of the substance in question in the aqueous phase divided by the concentration in the absorbent at equilibrium. These two partition coefficients are related by the thermodynamic equilibrium law. A very small gas-absorbent coefficient requires larger nutrient medium-absorbent coefficient (see Section 4.1.2.2). However, a big partition coefficient between the nutrient medium and the absorbent would have a negative influence on the overall elimination capacity of the system.

During periods of suboptimal biological degradation the toluene concentration in the culture medium would increase. Toluene would leave the reactor untreated through the aqueous instead of the gaseous phase.
The selected absorbent is silicone oil (AK50, Wacker GmbH, München, Germany), as it has small gas-absorbent partition coefficients for toluene and for oxygen. These oils have a high solubility for oxygen and are miscible with toluene. A rather low viscous silicone oil is chosen.

3.3.2 Properties

The dynamic viscosity of the absorbent is to 50 mPas, the density is 960 g/l and the oxygen solubility is in the range of 3.4 g/l, according to the manufacturer. The molecular weight of the selected silicone oil is 3'000 g/mol. The partition coefficients have to be experimentally determined, as described in Section 3.6.

3.3.3 Analysis methods

3.3.3.1 Oxygen

The dissolved oxygen (DO) concentration in the absorbent cannot be measured to an absolute value, due to the unknown solubility of oxygen in the chosen silicone oil. The oxygen measurement device allows only relative measurements. An oxygen Clark-Type probe P6100 with an oxygen transmitter 4100, delivered by Mettler Toledo (Uster, Switzerland), is used. The calibration is done by measuring silicone oil, free of oxygen, and silicone oil saturated with air. The gases used for the calibration are nitrogen (quality 5.0) and synthetic air featuring a concentration of 20% oxygen in nitrogen (quality 5.0), respectively (PanGas, Dagmarsellen, Switzerland). The sensor has been designed for measurements of dissolved oxygen in aqueous solutions. Therefore, the resulting oxygen concentration measurements can only be seen as an indication, not as an exact number of percent of saturation.
3.3.3.2 Toluene

The toluene concentration in the silicone oil is measured off-line by means of high performance liquid chromatography (HPLC) (Alliance 2690, Waters AG, Rupperswil, Switzerland) equipped with a Photodiode Analyzer (PDA) (PDA 996, Waters AG, Rupperswil, Switzerland). A packed phenyl-column (Nova-Pak Phenyl, 4 µm, 3.9 x 150 mm Cartridge, Waters AG, Rupperswil, Switzerland) with ethyl acetate (HPLC quality) as the mobile phase is employed to perform the analysis. The samples are run in an isocratic mode. The total flow rate is 1 ml/min. The detection wavelength is set to 260.5 nm. The analysis are carried out at ambient temperature. The silicone oil samples are taken using a glass syringe equipped with a PTFE plunger (Hamilton, Bonaduz, Switzerland) directly from the absorbent loop. Prior to the analysis, the samples are stored in 2 ml-HPLC glass vials sealed with PTFE lined silicone rubber septa (Infochroma GmbH, Grosshöchstetten, Switzerland).

3.3.3.3 Temperature

The temperature of the silicone oil is measured in the intermediate tank located above the biofilm modules by means of a Pt100 temperature sensor that is incorporated into the oxygen sensor (see Chapter 3.3.3.1).

3.4 Membranes

3.4.1 Selection

The membranes used to separate the gas, absorbent and aqueous medium are selected according to the following criteria: (i) the membrane has to separate the respective phases neatly, (ii) has to be applicable for a long-time experiment and (iii) the mass transfer from one phase to the other across the membrane should not decrease to a point where the removal of the VOCs is hindered.
An exact phase separation is important to the process due to the risk of cross-contamination of the two phases in contact with each other. A tested porous polypropylene (PP) membrane with a cut-off of 2'500 \( \text{g/mol} \) is not able to retain the oil at the pore mouths on the oil side of the membrane (data not shown). The (unintentional) application of transmembrane pressure differences may therefore lead to breakthrough (Poddar et al., 1996). This may occur on the absorbent side as well as on the gas side, forming an emulsion or bubbles in the silicone oil, respectively. Furthermore, the hydrophobicity of the PP leads to a substitution of the air in the pores by oil. Oil filled pores are undesired, because they would lead to an increased mass transfer resistance by forming a quiescent, diffusion controlled oil column in the pore. This resistance is of major influence, due to the large, average thickness of approximately 200 \( \mu \text{m} \) of porous membranes.

The same argumentation holds true for the separation of the aqueous culture medium from the absorbent. The absorbent may again enter the pore structure, or, when inverted, aqueous medium facing the porous side may lead to membrane fouling (Costerton et al., 1995). Bacteria increase the mass transfer resistance directly by growing within this structure and by clogging the pores, or indirectly by excretion of exopolymeric substances (EPS), that fill the pores. It can be concluded, that, in general, porous structures are not suitable for the separation of an aqueous phase containing microorganisms from an oily absorbent.

The solution to this problem is to use dense membranes, yet to maximize the mass transfer rate, they have to be as thin as possible. However, these dense membranes often feature a coarse, porous support structure for mechanical stability of the thin active membrane layer. Since porous structures are not applicable here (see above), a novel, ultra thin, dense poly dimethyl siloxane (PDMS) membrane (OPV-2551s-30n, CM-CELFA, Schwyz, Switzerland) is developed and applied.

### 3.4.2 Properties

The dense PDMS membrane is reinforced by a wire grating incorporated into the membrane structure for mechanical stability. The membrane has a total thickness of only 50 \( \mu \text{m} \). The grating is made of chromium steel.
wires each with a diameter of 18 \mu m. The distance between the square grids is 30 \mu m and the wire grid is calendered to a final thickness of only 30 \mu m. This means that at the grid junctions there is approximately 10 \mu m of PDMS on each side of the grid.

3.4.3 Mass transfer across and diffusion coefficients in the PDMS membrane

The overall mass transfer coefficient for toluene across the selected membrane is measured. This coefficient describes the transport from the bulk gas to the bulk absorbent. For oxygen, the overall mass transfer coefficient is calculated by means of the film theory, based on the experimentally determined diffusion coefficient in the membrane.

3.4.3.1 Film theory

The two-film theory is a useful model to describe mass transfer between (immiscible) phases (Grassmann et al., 1997). The idea is that a fluid-film or a mass-transfer boundary layer forms at the interface of the phases. According to the theory, a thin film of quiescent fluid exists on either side of the boundary; mass transfer is effected solely by molecular diffusion. In the case, where a dense membrane separates the two fluids, these boundary layers form on either side of the membrane, while the solute is also transported across the membrane by diffusion. A scheme of the film theory, including a phase separating membrane, is shown in Figure 3.1. The individual mass transfer resistances are combined to a single overall mass transfer coefficient by a resistance-in-series model approach, according to Equation 3.2:

\[
\frac{1}{k_{aSi}} = \frac{1}{\beta g_{Si}} + \frac{\delta_{memb}}{D_{memb}^{Si}} + \frac{1}{\beta a_{Si}}
\]

where \( \delta_{memb} \) denotes the thickness of the membrane, \( D_{memb}^{Si} \) the diffusion coefficient of the considered substrate in the membrane and \( \beta \) stands for the mass transfer coefficient caused by the boundary layers. This
Figure 3.1: Simplified concentration gradients from one phase to another across a dense membrane, based on the two film theory. The film resistances of the two fluids are included.

equation allows calculation of the diffusion coefficient $D_{\text{memb}}^{Si}$, based on a measurement of the overall mass transfer coefficient $k_{ov}^{aSi}$, as in the case of toluene. Vice versa it is possible to determine $k_{ov}^{aSi}$ if $D_{\text{memb}}^{Si}$ is known, as is done for oxygen. Both calculations require an estimation of the mass transfer coefficients $\beta$ induced by the boundary layers. A Sherwood ($Sh$) correlation for one-sided mass transfer in narrow channels is used. This correlation is adopted from a Nusselt ($Nu$) correlation (Stephan, 1960, Abb. 2.) by applying the Lewis analogy.

3.4.3.2 Mass transfer coefficient for toluene

The overall mass transfer coefficient for toluene is measured using a modified laboratory membrane test cell (P28, CM-CELFA, Schwyz, Switzerland). It has to be changed from a cross-flow filtration mode to a configuration which allows membrane based absorption. The absorbent is carried in the existing closed loop along the membrane, with the temperature of the absorbent kept constant. A gas stream loaded with a set concentration of toluene flows against the lower side of the horizontally mounted membrane. This flow rate is set sufficiently high to simulate an infinitely big reservoir with a steady concentration. The toluene concentration in the silicone oil is determined as a function of time. The overall mass transfer coefficient is calculated based on the increase of the
3.4. **MEMBRANES**

toluene concentration in the silicone oil with time. A mass balance for an ideally stirred bulk phase of the silicone oil is stated in Equation 3.3:

\[
\frac{\partial c^{aT}}{\partial t} = \frac{1}{V^a} \dot{M}^{g^a} = k_{ov}^{aT} \frac{A_{\text{memb}_{p28}}}{V_{ap28}} \left( \frac{1}{H^{gT}} e^{gT} - c^{aT}(t) \right). \tag{3.3}
\]

where \( c^{aT} \) denotes the concentration of toluene in the absorbent, \( V^{ap28} \) the volume of the silicone oil in the experiment, \( k_{ov}^{aT} \) the overall mass transfer coefficient for toluene based on the absorbent side, \( A_{\text{memb}_{p28}} \) the membrane area in the membrane test cell P28 and \( c^{gT} \) the concentration in the gas. It is important to note here, that the equation is derived for the bulk phase of the absorbent; it implies that the mass transfer resistance induced by the boundary layer is included in the overall mass transfer coefficient. The integration of Equation 3.3, introducing the substitution \( \Theta \), leads to linear Equation 3.4:

\[
-\ln(\Theta) = -\ln(\Theta_0) + k_{ov}^{aT} A_{\text{memb}_{p28}} \frac{1}{V_{ap28}} t \tag{3.4}
\]

Plotting \( \Theta \), calculated from the measured \( c^{aT} \) and the experimentally determined \( H^{gT} \) values, against the sampling time, allows the determination of \( k_{ov}^{aT} \) from the slope of a linear curve fit to the measured data points.

The temperature of the absorbent is set to 25°C. The gas contains toluene at a concentration of 5 g/m³ and has a flow rate of 1 l/min. The standard volume of silicone oil used for the experiment is 35 ml. The superficial velocity of the silicone oil in the meander of the support structure along the membrane is 0.2 cm/s. The membrane area is 28 cm² and the meander leading the silicone oil along the membrane is made of a channel with dimensions of 4 mm in width and 3 mm in height. The toluene concentration in the silicone oil is measured by HPLC analysis (see Chapter 3.3.3.2). The measurement is repeated threefold and three sample are obtained per time point. The calculated errors of the single measurements are combined using the rule of error propagation to give the total uncertainty of the averaged mass transfer coefficient.
3.4.3.3 Diffusion coefficient for oxygen

The method used to measure the apparent diffusion coefficient of oxygen in the dense PDMS membrane is a modification of the technique of Revsbech (1989a), which is based on relative dissolved oxygen profile measurements with oxygen microsensors.

The oxygen diffusion flux in a one-dimensional system, through a quiescent, homogeneous fluid or body, can be described by Fick’s first law (3.5):

\[ j = -D \frac{\partial c}{\partial z}. \]  

Under steady-state conditions the flux \( j \) is constant and directly proportional to the diffusion coefficient \( D \) and to the concentration gradient \( \partial c/\partial z \). The driving force to establish a flux is determined by the concentration difference of the adjoining fluids on either side of the body which is penetrated by diffusion. Placing two or more layers on top of each other, the flux through all layers is constant after reaching steady-state. The concentration profile along the thickness of the two layers is measured. The variation in the slope of the concentration profile (\( \partial c/\partial z \)) is therefore due to a change of the diffusion coefficient (\( D \)) in the two materials. If the diffusion coefficient is known in one of the two layers, the coefficient for the target body can be easily calculated based on the ratio of the slopes in the respective layers (3.6):

\[ D_{\text{memb}}^{O_2} \approx D_w^{O_2} \frac{\left( \frac{\Delta c^{O_2}}{\Delta z} \right)_w}{\left( \frac{\Delta c^{O_2}}{\Delta z} \right)_{\text{memb}}}. \]

The reference material of choice with a known diffusion coefficient is water, however since a quiescent water layer is difficult to achieve, an agarose gel is used. This gel is shown to have the same diffusion characteristics as water for a concentration range of 0.2 to 2% (w/v) if ‘skin’ formation by desiccation is avoided (Revsbech, 1989a). The measurements are carried out in a small diffusion chamber as illustrated in Figure 3.2. A sample of the membrane to be tested separates two chambers. The lower chamber holds a 0.1 M solution of the sodium salt of ascorbic acid. This is the anoxic side of the membrane due to the chemical reduction of all oxygen. An approximately 500 \( \mu \)m thick layer of 1% agarose is
Figure 3.2: Schematic drawing of the diffusion chamber for the determination of $D_{\text{memb}}^O_2$.

placed on top of the membrane. This agarose layer is left open to the air to assure oxygen saturation of the gel. The oxygen concentration profile through the agarose layer and through the membrane is acquired with an oxygen microsensor (Ox10, Unisense A/S, Aarhus, Denmark). The ratio of the two slopes allows the calculation of the diffusion coefficient in the dense PDMS membrane.

3.5 Nutrient solution

3.5.1 Composition

Minimal medium M9 (Sambrook and Russell, 2001) without an additional carbon source is chosen as the nutrient solution flowing over the biofilm. M9 contains the following salts dissolved in deionized water and is supplemented with the following amount of US* trace element solution (Panke et al., 1999).

Trace elements are added to the M9 medium because preliminary tests showed a yellowish color change, which indicates the production of siderophores due to a lack of iron. The composition of the US* trace element solution is given in Table 3.3.
Table 3.2: Composition of M9 mineral medium. For plate experiments, agar free of residual carbon sources is added.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$_2$HPO$_4$ · 7H$_2$O</td>
<td>32.00 g</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>7.50 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.25 g</td>
</tr>
<tr>
<td>NH$_4$Cl</td>
<td>2.50 g</td>
</tr>
<tr>
<td>US*</td>
<td>1.00 ml</td>
</tr>
<tr>
<td>Deionized water</td>
<td>add 1 l</td>
</tr>
<tr>
<td>Agar Noble</td>
<td>15.00 g</td>
</tr>
</tbody>
</table>

Table 3.3: Composition of US* trace element solution. The salts are dissolved in 1M hydrochloric acid.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>MnCl$_2$ · 4H$_2$O</td>
<td>1.50 g</td>
</tr>
<tr>
<td>ZnSO$_4$</td>
<td>1.05 g</td>
</tr>
<tr>
<td>H$_3$BO$_3$</td>
<td>0.30 g</td>
</tr>
<tr>
<td>Na$_2$MoO$_4$ · 2H$_2$O</td>
<td>0.25 g</td>
</tr>
<tr>
<td>CuCl$_2$ · 2H$_2$O</td>
<td>0.15 g</td>
</tr>
<tr>
<td>Na$_2$EDTA · 2H$_2$O</td>
<td>0.84 g</td>
</tr>
<tr>
<td>FeSO$_4$ · 7H$_2$O</td>
<td>4.87 g</td>
</tr>
<tr>
<td>CaCl$_2$ · 2H$_2$O</td>
<td>4.18 g</td>
</tr>
<tr>
<td>1M HCl(aq)</td>
<td>add 1 l</td>
</tr>
</tbody>
</table>

3.5.2 Analysis methods

3.5.2.1 Optical density

The optical density of the culture medium, samples from the lower tank, is measured daily at a wave length of 600 nm (Ultraspec 100pro, Amersham Biosciences, Otelfingen, Switzerland).
3.5.2.2 Toluene degradation products

The culture medium is analyzed for exemplary, known, stable intermediates produced during the degradation of toluene: benzyl alcohol, benzaldehyde, and benzoic acid, o-cresol (2-methyl phenol), m-cresol, p-cresol, p-hydroxy benzaldehyde, or p-hydroxy benzoic acid. The different forms of catechols are not included in this analysis procedure since they would cause the biofilm to turn blackish from polyphenol production and could therefore be detected by visual inspection. The presence of the previously referenced degradation products in the culture medium are measured off-line by means of HPLC (see Section 3.3.3.2 for the equipment). A packed C-18 column (Nova-Pak C_{18}, 3.9 x 150 mm, Waters AG, Rupperswil, Switzerland) is used with a mobile phase made of 20\%(v/v) acetonitrile and 80\%(v/v) trifluor acetic acid (TFA). The samples are run in an isocratic mode. The flow rate amounts to 1 ml/min. The detection wavelength is set to 210 nm. The retention times for benzyl alcohol, benzaldehyde, and benzoic acid, o-cresol (2-methyl phenol), m-cresol, p-cresol, p-hydroxy benzaldehyde, and p-hydroxy benzoic acid amount to 3.60, 7.01, 5.57, 5.82, 5.51, 5.69, 2.04 and 1.61 min, respectively. The run time is set to 30 min to elute and detect the above mentioned further degradation products. The analyses are carried out at ambient temperature. The liquid samples are taken from the lower medium tank with a glass syringe.

3.5.2.3 Dissolved O_{2}

The measurement of dissolved oxygen (DO) in the culture medium as well as the calibration of the sensor are carried out analogous to the procedure in the silicone oil (see Section 3.3.3.1). The probe is installed in the upper intermediate tank of the reactor.

3.5.2.4 pH

The pH of the medium is measured with a Polilyte HT 120 pH-probe (Hamilton, Dietrich + Blum AG, Wallisellen, Switzerland) connected
to a transmitter (Dulcometer D1C, Prominent Dosiertechnik AG, Regensdorf, Switzerland) and a converter (Dulcotest 4-20 mA, Prominent Dosiertechnik AG, Regensdorf, Switzerland). The probe is installed in the upper culture liquid tank besides the dissolved oxygen probe.

### 3.5.2.5 Dissolved CO₂

The dissolved CO₂ in the culture liquid is measured with a CO₂ probe InPro 5000 equipped with a transmitter 5100e (Mettler Toledo, Uster, Switzerland). The probe functions according to the Severinghaus principle (Severinghaus and Bradley, 1958); dissolved CO₂ diffuses through a membrane in the probe and changes the pH of a buffer solution within that probe. This pH change is analyzed and is shown to be proportional to the concentration of dissolved CO₂ in the aqueous sample (CO₂(aq)).

The CO₂ probe is installed in the upper intermediate tank next to the pH and the oxygen probe. The calibration is done analogous to the procedure used for the oxygen probe (see Section 3.3.3.1). The gas used for gauging is 8.01% of CO₂ in nitrogen (quality 5.0) (PanGas, Dagmersellen, Switzerland).

Depending on the pH of the aqueous solution, a significant part of the CO₂ may be present as carbonate – HCO₃⁻ or CO₃²⁻. This behavior is illustrated in Figure 3.3. According to Henry’s law the solubility of gaseous CO₂ in an aqueous solution is proportional to its partial pressure. The CO₂(aq) reacts with water to form carbonic acid, which again dissociates to a proton (H⁺) and hydrogen carbonate HCO₃⁻, for simplification only the pH range below 8 is considered:

\[
\begin{align*}
\text{CO}_2\text{(aq)} + \text{H}_2\text{O} & \rightleftharpoons \text{H}_2\text{CO}_3 \\
\text{H}_2\text{CO}_3 & \rightleftharpoons \text{H}^+ + \text{HCO}_3^- 
\end{align*}
\]  
(3.7a) (3.7b)

It is virtually impossible to distinguish analytically between CO₂(aq) and H₂CO₃, therefore the amount of carbonic acid is usually seen as the sum of both denoted H₂CO₅⁻. Only about 0.3% of the CO₂ available in the aqueous solution is H₂CO₃ and the remaining part is CO₂(aq), since the pKₐ₁ is 6.1 (the ”real” carbonic acid is a stronger acid with a pKₐ
3.5. NUTRIENT SOLUTION

Figure 3.3: The composition of a CO₂ water system as a function of the pH, according to the Henderson-Hasselbach Equation 3.8. The vertical line in the graph represents the pH prevailing in the real set-up.

The value of 3.8) (Stumm and Morgan, 1996). This equilibrium is established according to the Henderson-Hasselbach Equation:

\[
\text{pH} = \text{pK}_{a1} + \log \frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3^*]}
\] (3.8)

At pH 7 (the working pH of the bioreactor) approximately 90% (mol/mol) of all CO₂ in the liquid is present as HCO₃⁻. For a carbon balance if the reaction, this percent of the produced CO₂ must also be taken into consideration. The sensor only detects dissolved carbon dioxide CO₂(aq), therefore, the concentration of HCO₃⁻ has to be calculated based on the CO₂(aq) measurements shown in Equation 3.8.

3.5.2.6 Toluene

The toluene concentration in the nutrient medium is measured off-line by means of gas chromatography (GC) (Varian 2000, Waters AG, Rupperswil, Switzerland) equipped with a FID Detector. A capillary col-
umn (Zebron ZB-628 6% cyano propyl phenyl, 94% dimethyl polysiloxane, 30 m, 0.53 mm inner diameter, 3 μm film thickness, Brechbühler, Schlieren, Switzerland) with helium 5.0 as the carrier gas (PanGas, Darmstadt, Germany) is used. The analysis is done using ethanol as internal standard. The injector temperature is set to 120°C, the column pressure to 7 × 104 Pa. The temperature cycle run in the oven is as follows: 40°C for 2.5 min, then the temperature is increased to 70°C at 10°C/min. A further increase in temperature to 140°C at 20°C/min is carried out before ending with a holding period of 2 min. The retention time for ethanol and toluene is 1.9 and 7.3 min, respectively. The aqueous samples are taken directly from the nutrient medium loop of the reactor (the sampling and the storage procedure is analogous to the one in the silicone oil, referenced in Section 3.3.3.2).

3.5.2.7 Temperature

The temperature of the nutrient medium is measured on-line in the intermediate tank located above the biofilm modules by means of the oxygen probe, detailed in Section 3.3.3.1), which is equipped with a Pt100.

3.6 Partition coefficients

The partition coefficients for toluene and oxygen between the gas and the absorbent as well as between the absorbent and the aqueous phase have to be determined experimentally. These coefficients are important to establish when modeling the performance of the installation. The partition coefficients for the gas-absorbent as well as for the absorbent-water system are measured following the same process. The two phases are combined to achieve thermodynamical equilibrium. The partition coefficient is calculated as the concentration ratio of the related substrate between the two phases after reaching equilibrium. Equilibrium experiments are done for toluene and oxygen. The measurements are not done in the same set-up because of the experiment’s aim to lower the concentration in the analyzed phase by approximate a factor of two upon reaching equilibrium. The substrate is provided in one phase with a
defined concentration. After establishing equilibrium, the concentration in the same phase is measured by HPLC for toluene and by an oxygen probe for the dissolved oxygen. The concentration in the second phase is calculated by a simple mass balance. The experiments are carried out isothermally for three distinct temperatures. The analyses are done in triplicate and the concentrations measurements per sample are repeated three times.

3.6.1 Gas-absorbent

3.6.1.1 Partition coefficient for toluene

The partition coefficient measurements for toluene between air and the absorbent are done in 11 Erlenmeyer flasks with a ground neck. From previous experiments as well as from literature (Poddar and Sirkar, 1996) it is known that the partition coefficient $H_{gaT}$, defined as $c_g^T/c_a^T$, is in the range of $10^{-3}$. Therefore, since the concentration of toluene in the silicone oil should be decreased by a factor of two, once equilibrium has been reached, the volume of silicone oil is set to 1 ml. The silicone oil, featuring a known distinct concentration of toluene, is filled in a HPLC glass vial and placed into an Erlenmeyer flask. The silicone oil is stirred using a micro-magnetic stirrer in the vial. The partition coefficients are measured for three concentrations, 5’000, 10’000 and 20’000 g/m³ of toluene in the silicone oil. The flasks holding the HPLC vial are placed in a cryostat to keep the temperature constant at 20 °C, 30 °C and 40 °C for the individual experiments. Equilibrium is shown to be reached after approximately 40 h, thereafter, to ensure equilibrium, the samples are left for an average of three days.

3.6.1.2 Partition coefficient for oxygen

The partition coefficient for oxygen between gas and absorbent is measured in a round bottom flask holding a total volume of 100 ml. The oxygen concentration measurements are done in the gas phase by means of an oxygen microsensor (described in more details in Chapter 3.9.4).
The microsensor is sealed to the flask and against the environment using a teflon sealing. The sensor is known to consume very little oxygen. Preliminary experiments indicated that the dimensionless partition coefficient $H^{O_2}_{g\alpha}$, defined as $c^{gO_2}/c^{\alpha O_2}$, is in the range of 5. Therefore, 50 ml of anoxic silicone oil, stripped for at least 10 min with nitrogen by vigorous bubbling, are pipetted into the round bottom flask which has previously been flushed with air to ensure a constant atmospheric oxygen concentration. The oxygen concentration in the air is recorded as a function of time. The partition coefficient experiments are carried out at a fixed temperature of 25 °C in a cryostat bath. The use of a magnetic stirrer in the silicone oil is omitted, due to the fragility of the filigree oxygen sensor tip.

3.6.2 Absorbent-water

3.6.2.1 Partition coefficient for toluene

The partition coefficients for toluene between the absorbent and water are analyzed using the same procedure as in Section 3.6.1.1 describing the equilibrium between gas and absorbent. As preliminary experiments indicated, the coefficient $H^{amT}$, defined as the absorbent concentration divided by the toluene concentration of the aqueous phase $c^{aT}/c^{mT}$, is in the range of $4 \cdot 10^{-3}$ as preliminary experiments showed. Therefore, smaller Erlenmeyer flasks holding only a volume of 200 ml are used. The flasks with a ground neck are stirred using magnetic stirrers and the temperature is fixed in a cryostat bath. The experiments are carried out at temperatures of 20 and 40 °C with starting toluene concentrations in the silicone oil of 5'000 and 10'000 g/m³. The volume ratio of water to silicone oil is set for all experiments to 46. At the end of the experiment the partly emulsified mixture is left to separate for one day, before the concentration of toluene in the silicone oil is analyzed.

3.6.2.2 Partition coefficient for oxygen

The analysis of the partition coefficient for oxygen in a silicone oil water two-phase system is done analogous to the analysis of the gas absorbent
system in a 35 ml round flask bottle. The oxygen concentration is measured in the aqueous phase. 30 ml of deionized water in the round bottom flask are saturated with oxygen by bubbling with air. During this operation the flask is partly submerged in the water bath and the temperature is kept constant at 25°C. 5 ml of anoxic silicone oil are added to the flask, which is tightly sealed and then completely submerged in the water bath.

### 3.7 Data acquisition

All determined on-line data is acquired using two data acquisition devices (DAQ) (AT-MIO 16 XE-50), one equipped with a connection box (SCB-68) the other with a multiplexer (SCXI-1100, all devices National Instruments, Ennetbaden, Switzerland). The information is averaged and logged by means of an automation and measurement software (LabView 7.1, NI), acquired at a rate of 1 Hz and averaged over a period of 60 s.

### 3.8 Inoculum

Part of the project aims at analyzing the composition of the toluene degrading bacterial consortium in the biofilm as a function of time. For this investigation a molecular technique (Polymerase Chain Reaction (PCR); see Chapter 3.9.1.7) is applied. This allows the identification of specific, previously known strains, but not the identification of all microorganisms.

#### 3.8.1 Strains for inoculation

The two strains used for inoculating the reactor are *Pseudomonas putida F1* and *Rhodococcus globerulus PWD1*. Both bacteria are able to use aromatic hydrocarbons, such as toluene, as their sole energy and carbon source.
3.8.1.1 *Pseudomonas putida* F1

Bacteria belonging to the *Pseudomonas* group are common inhabitants of soil and water but can also be found on the surface of plants and animals. These bacteria exist in nature in a biofilm and in planktonic form. The latter can display an extremely high motility due to its polar flagella. *Pseudomonas* are renowned for their metabolic versatility as they can grow under a variety of growth conditions and do not require any stimulation factors.

*Pseudomonas putida* share a high degree of homology to *Pseudomonas aeruginosa* strains. However, key virulence factors like certain exotoxin genes and type III secretion systems are absent, making them non-pathogenic (Prescott et al., 1996). Since they are metabolically versatile, and well characterized, they are very good candidates for biocatalysis or bioremediation.

*P. putida* F1 (ATCC 700007, DSMZ 6899) also referred to as Trevisan 1889, Migula 1895AL or *Arthrobacter siderocapsulatus*. *P. putida* F1 is a rod shaped, single arranged, motile, aerobic, gram-negative bacterium which grows in multiple habitats at mesophilic temperatures. The strain is resistant to solvents (Huertas et al., 1998), it is even able to grow in solvent-water two phase systems that contain up to 50%(v/v) solvents, which feature logP<sub>OCT</sub> values typically higher than approximately 2.5 (Cruden et al., 1992). Toluene has a logP<sub>OCT</sub> value between 2.5 (Cruden et al., 1992) and 2.7 (Eastcott et al., 1988), yet *P. putida* F1 cannot grow in aqueous/toluene two phase systems (Cruden et al., 1992). *P. putida* F1 is able to metabolize a wide range of aromatic compounds such as toluene, o-cresol, m-cresol (Spain and Gibson, 1988) or p-cresol (Cruden et al., 1992). Chlorinated benzenes and phenols are degraded to chlorinated catechols. These compounds accumulate in the culture medium due to the inability of *P. putida* F1 to degrade chlorinated catechols.

**Degradation pathway for toluene** The oxidative degradation of aromatic hydrocarbons to catechols by *P. putida* F1 was first described by Gibson et al. (1968). *P. putida* F1 degrades toluene via cis-toluene di-
3.8. INOCULUM

Hydrodiol to 3-methylcatechol (see Figure 3.4). The first four steps in the pathway involve the sequential action of toluene dioxygenase (encoded by todABC1C2), cis-dihydrodiol dehydrogenase (todD), 3-methylcatechol 2,3-dioxygenase (todE) and 2-hydroxy-6-oxo-2,4-heptadienoate hydrolase (todF). The genes for these enzymes form part of the tod operon which is responsible for the degradation of toluene by this organism. The physical order of these genes is todF, todC1, todC2, todB, todA, todD, todE (Zylstra et al., 1988). The primers for the PCR test (see Chapter 3.9.1.7) are designed against the todC1 gene, which encodes the toluene dioxygenase.

3.8.1.2 *Rhodococcus globerulus* PWD1

The bacterium *Rhodococcus globerulus* PWD1 (NCBI 13325) is a soil isolate from a polluted site in the Netherlands. It is an elongated curved, rod shaped, gram-positive bacterium (Barnes et al., 1997).

**Degradation pathway for toluene**  *R. globerulus* PWD1 is able to degrade a broad range of aromatic compounds, including toluene (Barnes et al., 1997). However, the exact degradation pathway for this organism is not known. It is believed to follow the same path as *P. putida* F1 (Barnes et al., 1997). This is assumed based on two results which are identical for *P. putida* F1 as well as for *R. globerulus* PWD1. Firstly, there is good growth on toluene, ethylbenzene and benzene and no growth on m-xylene or p-xylene; secondly, the colonies turned yellow after exposure to phenylacetylene (Duetz, 2004). However, Cruden et al. (1992) did report *P. putida* F1 able to degrade p-xylene.

**Sequenced genome**  The gene clusters appearing to encode for the pathway of the oxidative degradation of a phenolic acid, 3-(3-hydroxyphenyl) propionate (3hpp), to 2-keto-4-pentenoic acid have been sequenced (Barnes et al., 1997). The first three steps in the pathway involve the sequential action of a hydrolase (hppA), an extradiol dioxygenase (hppB) and a hydroxymuconic semialdehyde hydrolase (hppC). The physical order of these genes is hppA, hppB, hppC (Barnes
et al., 1997).
The primers for the PCR test (see Chapter 3.9.1.7) are designed against the hppB and the adjacent hppK gene, located on the hpp operon.

3.8.2 Degradation pathways for toluene

**Aerobic degradation**  Toluene is readily degraded by bacteria following aerobic as well as anaerobic pathways. The aerobic toluene degrading bacteria follow basically two strategies: Initial attack can either occur at the side chain or at the aromatic ring. Typical intermediates are benzoic acid, catechols and semialdehydes (see Figure 3.4) (Mikesell et al., 1993; Wackett, 2004). These two strategies account for a total of five demonstrated pathways for the aerobic metabolism of toluene (Mikesell et al., 1993). There are three pathways resulting in 3-methylcatechol; one with dioxygenation at the 2,3 positions of the benzene ring, the other two with initial hydroxylations at the 2 and at the 3 position. The other two known pathways follow the strategy of oxidizing the methyl group (Mikesell et al., 1993). One pathway directly oxidizes the methyl group to give benzoic acid and finally catechol. The other requires a hydroxylation of the benzene ring at the 4 position prior to an oxidation of the methyl group. It is interesting to note that in the latter case the formation of (4-hydroxy) benzyl alcohol is circumvented and (4-hydroxy) benzaldehyde is formed directly, to finally form 2,4-dihydroxybenzoic acid.
Figure 3.4: The known aerobic bacterial degradation pathways of toluene.
Anaerobic degradation Anaerobic toluene mineralization under denitrifying conditions has been demonstrated for pure bacterial cultures (Altenschmidt and Fuchs, 1991; Dolfing et al., 1990; Evans et al., 1991; Schocher et al., 1991) (see Figure 3.5). There is even a report that describes pure bacterial cultures able to utilize toluene under iron-reducing conditions (Lovley and Lonergan, 1990). The anaerobic degradation pathway under denitrifying conditions is initiated by oxidation of the methyl group to form benzylsuccinate. The formation of benzylsuccinate from toluene is independent of coenzyme A and nitrate, but it requires the presence of fumarate as electron acceptor. Benzylsuccinate is further oxidized via E-phenylitaconyl-CoA, and benzoyl-CoA, to benzoate. These oxidation steps require the presence of coenzyme A and nitrate (Beller and Spormann, 1997; Biegert et al., 1996).

![Figure 3.5: The known anaerobic bacterial degradation pathways of toluene under denitrifying conditions.](image)

3.8.3 Cultivation and inoculation procedure

LB plates are inoculated under sterile conditions with *P. putida* F1 and *R. globerulus* PWD1, respectively, from glycerol stocks kept at \(-80^\circ\text{C}\). Single colonies are picked and transferred to test tubes loaded with 4 ml of liquid LB medium. 2 ml each of grown pre-culture, *P. putida* and *R. globerulus*, are used as inoculum for distinct minimal medium (M9).
3.9 Biological investigations

A good approach to investigate biofilms should include three strategies:

- one technique able to give information about the microbial species active in the biofilm
CHAPTER 3. MATERIALS AND METHODS

- a method that gives spatial information, e.g. microscopy, to visualize the biofilm structure (Surman et al., 1996), biofilm surface scanning (Vinage, 2002) or microsensor analyses (Revsbech, 1989b))

- and procedure that gives information on the physiological parameters (e.g. maximum specific growth rate or yields).

3.9.1 Microorganism identification

3.9.1.1 Introduction

In nature, biofilms combine a broad range of living organisms, such as bacteria, fungi and protozoa (??). Model set-ups, which grow biofilms in vitro, can either be run monoseptically or open. For the monoseptical biofilm reactors the inoculating microorganisms are usually defined. Often, very little information about the microbial consortia is provided for open systems. Sometimes the exact inoculating strains are stated (e.g. Vinage, 2002) but very few studies have been carried out investigating the microbiological composition of biofilms - with or without known composition of the inoculum - as a function of time.

In this study one focus is set on the bacterial composition of the biofilm. The existing reactor is run non-monoseptically and is inoculated with two known toluene degrading strains (see Chapter 3.8.1). The question is whether one of these two strains will establish itself and whether a biofilm of one or several different toluene degrading bacterial strains emerges. Bacteria, which are not able to degrade toluene, are not characterized. To analyze toluene degraders, a combination of plate selection and a molecular technique is applied.

3.9.1.2 Principles of bacteria plating

There are two methods for bacterial plating: streak plating and spread (or dilution) plating. The principle of streak plating holds, that individual microbial cells can be separated by dragging (streaking) very few
cells over the surface of the agar, which then grow during incubation into individual colonies. This technique indicates whether a culture is viable or pure.

Spread plating is used to identify the number of viable microorganisms in a given amount of liquid. Serial dilutions are made, and fixed amounts of these dilutions are plated on an appropriate agar and incubated, from which different numbers of colonies will be obtained. Based on this data, the number of microorganisms in the original source culture can be calculated.

3.9.1.3 Principle of Polymerase Chain Reaction (PCR)

PCR is a laboratory method for copying specific, (partially) known DNA sequences. The process begins by separating double stranded DNA into two single strands by heating to 94°C. At the beginning and end of the specific sequence, so called primers anneal after a temperature change. These primers are complementary to the DNA that is supposed to be amplified. After a third temperature change, a DNA polymerase synthesizes the DNA between the two primers. The resulting new double stranded DNA pieces are melted again by increasing the temperature. This frees the binding sites of the two primers and after lowering the temperature the process can be restarted. Completing this temperature cycle results in a doubling of the DNA sequence in question. Repeating this procedure causes an exponential amplification of the DNA sequence, which are analyzed by gel electrophoresis.

3.9.1.4 Analysis Procedure

A simple procedure to investigate living, aerobic culturable bacterial strains in the biofilm is used. A resuspended biofilm sample is plated on Luria-Bertani (LB) agar, to give the total number of colony forming units (CFU). The percentage of toluene degraders of all culturable colonies is determined by plate selection. The bacteria are transferred one by one to a minimal medium plate with toluene in the gas-phase as the only carbon (C-) source. Only bacteria that can use toluene as C-source and energy
source will grow on this second plate. These toluene degrading bacteria are differentiated by use of PCR. Specific primer sets will differentiate \textit{P. putida} F1 and \textit{R. globerulus} PWD1 from each other and the rest of the toluene degraders. The latter group is not further differentiated.

### 3.9.1.5 Plate count

A sample of the biofilm is taken during the operation of the reactor. This is done with a cylindrical sampling device (inner diameter 25 mm) directly from the membrane. This sampling device features two lip sealings at the front end; one crosses the tube diagonally, the other is mounted in the extension of the tube. By slightly pressing this device against the membrane surface and turning it several times around its longitudinal axis a distinct area of the biofilm, used for analysis, is separated and detached by the diagonal lip seal. The separated biofilm with the culture liquid is pipetted to a 2 ml-Safe Lock vial (Eppendorf, Hamburg, Germany). The mixture of sloughed biofilm and supernatant is homogenized by vortexing (Vortex-Genie 2, Model G560E, Scientific Industries, Bohemia, U.S.A.) at maximum speed for 1 min. Dilution series are made with this cell-suspension in triplicate. 100 \( \mu l \) of the dilutions 1 : 10\(^5\) and 1 : 10\(^6\) are spread in triplicate on LB agar plates (see Table 3.4). The counts of these CFU are used as the total viable number of aerobe, culturable bacteria, per area of biofilm.

<table>
<thead>
<tr>
<th>Table 3.4: Composition of the LB medium with the addition of agar.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
</tr>
<tr>
<td>Yeast extract</td>
</tr>
<tr>
<td>NaCl</td>
</tr>
<tr>
<td>Deionized water</td>
</tr>
<tr>
<td>Bacto Agar</td>
</tr>
</tbody>
</table>
3.9. BIOLOGICAL INVESTIGATIONS

3.9.1.6 Plate selection

An average number of 50 randomly chosen cells are picked from the master plate, containing all viable bacteria growing on LB-medium. With sterile toothpicks, cell material from distinct colonies is successively transferred to two M9 minimal medium plates and – finally – to an LB plate. The first M9 plate is incubated without an additional carbon source. The second plate is placed in a desiccator, where it is exposed to toluene vapor. The desiccator holds a total volume of approximately 101. The exsiccator contains a glass beaker with approximately 250 ml of water containing toluene. The initial concentration of toluene in the water is 0.2 g/l for the first 24h. The maximum initial toluene concentration in the gas phase is with that 4.5 g/m³; it results from a simple mass balance for the volume of the desiccator. The maximum theoretical concentration in the plates reaches therefore 17.3 g/m³ ($H_{gmT} = 0.26$). Starting from day two, the concentration is lowered to 0.1 g/l. This aqueous solution is replaced daily or upon opening the desiccator (Deshusses, 2004). All plates are incubated at room temperature.

Growth under different conditions is evaluated. The plate without C-source serves as negative control, to check whether there are impurities of carbon in the agar or whether there is carbon from the LB plates dragged along with the cells. The number of colonies grown on the M9 plate with toluene as C-source, serves to calculate the ratio of all aerobic, culturable bacteria that are able to grow on toluene. The LB plate at the end serves as the positive control, to check whether sufficient cells remained on the toothpick to inoculate the two previous plates.

3.9.1.7 PCR-test

The differentiation of the percentage of the toluene degrading bacteria is done using a colony-PCR test. Two specific sets of PCR primers are designed, one for each inoculated strain, P. putida F1 and R. globetulbus PWD1. By using PCR two specific, distinguishable and known DNA sequences are amplified. This enables the differentiation of the two inoculated strains from each other, as well as the separation from newly recruited toluene degraders.
The sequences for forward and reverse primers of \textit{P. putida} F1 are: 5'-GAC CCC AAT CTG ATG CTT GCC-3' and 5'-TGT CAT CAT CCG CAG CCA ATG-3', respectively. They anneal to parts of the \textit{todC1} gene of the \textit{tod} operon (Zylstra et al. (1988), Genbank source file: >gi|J04996) and produce a 513 bp piece of DNA after PCR. The chosen gene sequence of the \textit{R. globerulus} PWD1 strain is the \textit{hpp} operon (hydroxyphenyl propionate operon). This operon was sequenced by Barnes et al. (1997) (Genbank source file: >gi|1905989). The forward and the reverse primers read as follows: 5'-ATC GGTGGAAGC AGC GTTCG-3' and 5'-GTC GAT CAT GCT GAG GAT CAG-3', respectively. The resulting DNA fragment is 1011 bp long. All primers are designed using the Lasergene v6 (PrimerSelect) software (DNASTAR, GATC Biotech AG, Konstanz, Germany) and obtained from Microsynth AG (Balgach, Switzerland).

Colony PCR is carried out by suspending cells from a single colony in 50 \(\mu\text{l}\) sterile, deionized water (ddH\(\text{O}\)) in a 1.5 ml-Safe Lock vial (Eppendorf, Hamburg, Germany). Cells are lysed by adding 10\%(v/v) chloroform followed by vigorous vortexing for 1 min. This two-phase mixture is separated by centrifugation for 5 min at 16'000g (centrifuge 5414D, Eppendorf, Hamburg, Germany). Cell debris is collected together with the chloroform phase at the bottom of the vial. 30 \(\mu\text{l}\) of the supernatant containing the chromosomal DNA are transferred to a clean vial and can serve as template for the PCR.

The PCR test is done separately for \textit{P. putida} F1 and for \textit{R. globerulus} PWD1. Multiplex PCR could not be applied due to different, individually optimized PCR reaction mixtures for the two strains. The amplification is performed in 20 \(\mu\text{l}\) reactions (0.2 ml PCR tubes, Eppendorf, Hamburg, Germany). PCR mixtures for the two tested strains are listed in Table 3.5. KCl-Buffer (10X Taq KCL-Buffer) and MgCl\(_2\)-solution (25 mM MgCl\(_2\)) are purchased form Fermentas GmbH (c/o Labforce AG, Nunningen, Switzerland). Deoxy-Nucleotides (dNTPs) are obtained from Roche Diagnostics (Rotkreuz, Switzerland). A set consisted of 100 mM aqueous solutions of dATP, dCTP, dGTP and dTTP each in a separate vial. The four nucleotide solutions are mixed (10 mM each dNTP) and stored as aliquots at \(-20\, ^\circ \text{C}\). Taq Polymerase is delivered by New England BioLabs (c/o Bioconcept, Allschwil, Switzerland) at a concentration of 5.0 units/\(\mu\text{l}\). All reagents are briefly vortexed and centrifuged.
3.9. BIOLOGICAL INVESTIGATIONS

upon thawing before use. The PCR master-mix is prepared on ice according to the recipe listed in Table 3.5. The DNA template is present in

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**Table 3.5:** Compositions of the PCR mixes for *P. putida* F1 and for *R. globularus* PWD1. The addition of the reagents to the master mix is effected in the same order as stated in the list above.

<table>
<thead>
<tr>
<th></th>
<th><em>P. putida</em> F1</th>
<th><em>R. globularus</em> PWD1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Template</strong></td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>sterile ddH2O</td>
<td>9.82</td>
<td>9.42</td>
</tr>
<tr>
<td>KCl-Buffer</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Primer forward (10 μM)</td>
<td>0.80</td>
<td>0.80</td>
</tr>
<tr>
<td>Primer reverse (10 μM)</td>
<td>0.80</td>
<td>0.80</td>
</tr>
<tr>
<td>dNTPs (10 mM)</td>
<td>0.30</td>
<td>0.50</td>
</tr>
<tr>
<td>MgCl2 (25 mM)</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Taq Polymerase (5.0 units/μl)</td>
<td>0.28</td>
<td>0.28</td>
</tr>
</tbody>
</table>

The PCR vial when the master-mix is added. The solution is briefly centrifuged before starting the thermal cycle. The employed thermal cycler protocol is the same for both tested strains. The lid of the thermal cycler (Mastercycler Eppendorf, Vaudaux-Eppendorf, Schwarzenbach, Switzerland) is preheated to 105°C. The DNA is denatured at 94°C for 2 min, and the fragments are amplified during 32 cycles of 1 min at 94°C, 30 s at 52°C and 1.5 min at 72°C. A final elongation for 10 min at 72°C is added before the mixture is cooled down to and stored at 4°C. All temperature changes are accomplished at a rate of 0.3 °C/s. Each sample is tested in duplicate; two independently made master-mixes are used with the same DNA template. In all experiments, appropriate negative controls containing no template DNA but water are subjected to the same procedure. The PCR positive controls are done with strains starting from LB plates, which are inoculated with cells of the two tested strains (glycerol stocks, stored at −80°C). All reactions are analyzed by agarose gel electrophoresis (2% (w/v) agarose (GTQ, Carl Roth GmbH, Karlsruhe, Germany) in 0.5% TAE (tris acetic acid), which is also used
as running buffer for the electrophoresis carried out at 80 V. In order to visualize the DNA bands later under UV-light, 2 μl Ethidium Bromide (stock solution 1 g/ml Fluka, Balgach, Switzerland) are added to 30 ml of melted Agarose.

3.9.2 Confocal Laser Scanning Microscopy observation of the biofilm

3.9.2.1 Introduction

The structures in biofilms can be analyzed in three different states: dehydrated, partly dehydrated and hydrated. Dehydration is a prerequisite for electron microscopy (EM) techniques. The information is cross-sectional, including cell location and internal cell structures, yet the big disadvantage of EM methods is the shrinking due to dehydration and fixing of the samples. For example, the EPS matrix in biofilms will shrink to a fraction of the original volume, leading to a loss of 3D information and making it difficult if not impossible to draw conclusions regarding the original biofilm state (Neu et al., 2001).

Fully hydrated biofilms can be examined via light microscopy techniques, but normal light microscopy is limited to samples just a few micrometers thick. Because biofilms may achieve a few hundred micrometers in thickness, different techniques are necessary to visualize the 3D structure, e.g. confocal laser scanning microscopy (CFLSM).

3.9.2.2 Principle of Confocal Laser Scanning Microscopy (CFLSM)

CFLSM is a combination of traditional epifluorescence microscopy with a laser light source, specialized scanning equipment and digital imaging. The key advantage is the possibility to observe thin optical sections in thick, intact specimens. In conventional fluorescence microscopy, out-of-focus fluorescence, evoked by the excitation light, tends to overwhelm details in the actual image plane. CFLSM uses a tiny laser spot, focused in a defined image plane, to excite fluorescence. This spot is scanned
in lines across the field of view, resembling image formation by an electron beam in a TV-screen. The scanning unit "writes" the laser spot along columns and lines into the image plane. Detection of fluorescence is done by a high sensitivity photomultiplier. Out-of-focus fluorescence will not contribute to the final image, this is achieved in two ways: first, by illuminating only one point, and second by excluding any remaining fluorescence that is not in the focal plane with a detector pinhole (see Lawrence and Neu (1999) for a schematic of a confocal laser scanning microscope). A laser produces a high-intensity, coherent light beam of a defined wavelength, which is deflected by a dichroic mirror (beam splitter) into the objective. The objective projects (focuses) this beam into the focal plane within the specimen. A mixture of emitted fluorescent light as well as reflected laser light from the illuminated spot is then collected by the objective lens. A beam splitter separates the light mixture by reflecting the laser light and allowing only the fluorescent light to pass into the detection apparatus. After passing a pinhole, which is in a confocal position (conjugate) to the excitation pinhole, the fluorescent light is detected by a photo-detection device (photomultiplier tube) transforming the light signal into an electrical one which is recorded by a computer (Pawley, 1995).

The key step for the successful application of CFLSM is the staining of the specimen. The fluorescent stain specifies the application (McFeters et al., 1995) e.g. detection of microorganisms in a fully hydrated, living biofilm (Lawrence et al., 1991), or in-situ localization of exopolymers in biofilms (Wolfaardt et al., 1998).

3.9.2.3 CFLSM and digital image analysis

An inverted CFLSM Leica TCS SP1 mounted on a Leica DM/RE (Leica, Mannheim, Germany) is used to acquire image stacks of the biofilm. An argon laser, emitting at 488 nm, is used as the excitation source. The microscope is equipped with oil immersion lenses (10x0.4 numerical aperture (NA) or, 20x0.7 NA). The Leica Confocal Software is used for the operation of the system and for the analysis of the digitized images. The biofilm is sectionally analyzed by the inverted microscope, through the transparent membrane. For this purpose, a section of the membrane
covered with biofilm has to be cut out. This implies that the CFLSM-investigations can only be accomplished after shutting down the reactor. Sectioning is done by acquiring data from slices of 1 μm thickness. Due to the metallic grid incorporated into the membrane (see Chapter 3.4), the optical access is not feasible for the whole area of the biofilm. Therefore, the regions of interest (ROIs) have to be drawn manually to the images. The ROIs, drawn in the clearances of the grid, sample for the fluorescent information of the biofilm and the ones, drawn on the nodal points, sample for the background fluorescence. An example of such an image is shown in Figure 3.6. To carry out the analysis, as many ROIs as possible are drawn to the areas on the picture, which are undisturbed by the grid. The fluorescent intensity of the background, which has to be subtracted from each individual picture, is determined by averaging.

**Figure 3.6**: CFLSM image of a stained biofilm. The lines in the picture represent the zones in which the optical information is extincted by the grid in the membrane. The circles mark the ROIs, which are analyzed for fluorescent intensities. The picture is acquired at a z-position below the metallic gird, incorporated in the membrane (the bar represents 50 μm).
five ROIs placed on the nodal grid points.

### 3.9.2.4 Distribution of live/dead bacteria in the biofilm

The differentiation of live and dead microorganisms in the biofilm is done by using the LIVE/DEAD® BacLight™ Bacterial Viability Kit from Molecular Probes, Inc. (Invitrogen, Basel, Switzerland). This kit employs two nucleic acid stains - the green-fluorescent SYTO9 and the red-fluorescent propidium iodide (PI) stain. Although this staining system is intended for use with pure cultures grown as planktonic cells, it was successfully applied for biofilm cells (e.g., Neu and Lawrence, 1997; Takenaka et al., 2001). Circular pieces with a diameter of 20 mm are cut for analysis from a biofilm covered membrane that has just been removed from the reactor. The living bacteria are stained for 15 min with 600 µl of a mixture made of 1 µl of 3.34 µM SYTO9 in dimethylsulfoxide (DMSO) diluted with 1 ml of phosphate buffer saline (PBS) (pH 7.0). The dead organisms are stained with 600 µl of a solution of 1 µl 20.0 µM PI in DMSO dissolved in 1 ml of PBS. Both stains are applied at the same time to the same sample. SYTO9 penetrates all bacterial membranes and labels both live and dead bacteria. In contrast, PI penetrates only bacteria with damaged membranes. Thus, living bacteria with intact membranes fluoresce green, while dead bacteria with damaged membranes fluoresce red (and green). The ratio of both fluorescence intensities specifies the proportion of live to dead organisms. The probes are subsequently washed 3 times with 1 ml PBS to remove residual, unbound fluorescent stain from the biofilm. All samples stained in this fashion are immediately examined by CFLSM. Both stains are excited by the laser, emitting light at a wavelength of 488 nm. The emissions of SYTO9 and PI peak at 530 nm and 605 nm, respectively and can be clearly separated using the appropriate detector settings.

For each microscopically scanned biofilm sample, there are up to 17 ROIs analyzed. These ROIs add up to an average investigated area of 13’000 µm². The depth of analysis is determined by the measurable intensity. At an averaged biofilm depth of approximately 100 µm, the intensity of the signal drops to a constant level equal to the background intensity. The intensity values of all ROIs for each single biofilm level,
are averaged and the standard deviation is calculated. These averaged green and red intensities are divided by each other for each level in the biofilm, allowing the calculation of the live to dead ratio from an appropriate calibration curve.

During CFLSM the precise position within the biofilm is not exactly known due to a lack of a definite lead. Therefore, the measurements are started at a z-position at what is assumed to see the undersurface of the membrane in reference to the biofilm, identified by the reflection of the metallic grid in the membrane (see Figure 3.6). However, this starting position cannot be exactly maintained at all times. The acquisition of the intensities starts below, within or above the membrane, already in the biofilm. This means, that the measured intensity profiles have to be axially displaced against each other along the abscissa until they overlap (see Figure 3.7). At the overlapping band the curves are averaged, calculating the arithmetic average, whereas the average errors are calculated using the quadratic sum (Taylor, 1997). The first, increasing part of the curve is omitted, due to the fact that it is within or below the

Figure 3.7: Two example plots of CFLSM analysis, showing the green to red intensity ratios. The two curves are displaced against each other and overlayed. The data points in the first increasing part of the curve are acquired within the membrane.
membrane. The boundary between the membrane and the biofilm is set to the peak position of the intensity curve (see Figure 3.7).

The percentage of live to dead bacteria in the biofilm is calculated from the intensity ratio. A calibration curve is made plotting the fluorescent intensity ratio versus the known percentage of live bacteria. A two-point calibration curve is done using a planktonic cell culture: 100% of dead bacteria and 100% alive. The cells are grown in a Biochemical Oxygen Demand (BOD) Measuring System (see Section 3.9.6 for detailed information) to assure cells are harvested from an exponentially growing culture. 50ml are spun down at 16,100 g for 10 min and resuspended in 10 ml mineral medium (M9). This solution serves as the 100% live sample. 1 ml of this culture is pipetted to a 2ml-Safe Lock vial (Eppendorf, Hamburg, Germany) where the bacteria in suspension are killed by adding 10%(v/v) toluene (De Smet et al., 1978; Sikkema et al., 1994) and gently shaking for 15 min at 30 °C. The bacteria are killed due to a partial disintegration of the cytoplasmic membrane. The cell walls are punctured, forming a porous membrane with a cut-off at 50kDa (De Smet et al., 1978). After a subsequent centrifugation at 16,100 g for 3 min, and the removal of the supernatant, the perforated cells are resuspended in 0.8 ml PBS. This suspension serves as the dead sample.

3.9.2.5 Distribution of Exopolymeric substances in the biofilm

The extracellular matrix is built of metabolites excreted from the bacterial cells as well as from organic and inorganic components from the nutrient medium (Jahn et al., 1999). This structural matrix enmeshing the communities is mostly composed of structured exopolysaccharides (EPS) (Read and Costerton, 1987). All bacteria growing in nature or pathogenic environments produce, at their cell surfaces, an exopolysaccharide glycocalyx. This glycocalyx surrounds the bacterial cell and consists of highly hydrated polyanionic polymers, which serve as an ion exchange resin (Costerton, 1999). This matrix determines important properties of a biofilm. The EPS mediates the adhesion of bacterial cells to surfaces and due to its ion exchange properties it traps and concentrates nutrients in the biofilm and it protects the cells within the biofilm from antibiotics and bacteriocidal substances (e.g. toluene).
These agents must first saturate and penetrate the biofilm matrix before reaching the bacterial cells. All these inherent properties make knowledge of the EPS distribution within the biofilm highly important.

The presence and distribution of EPS can be investigated by chemical or microscopic means (Strathmann et al., 2002). Chemical techniques involve an extraction of the EPS, which destroy the biofilm and do not give information about the spatial distribution. In contrast CFLSM together with the application of fluorescent probes can be used for visualization and quantification of certain biofilm components (Lawrence et al., 1998; Neu and Lawrence, 1999).

Labeled lectins are used as fluorescent probes for the investigation of the EPS in biofilms by CFLSM (e.g. Neu and Lawrence, 1997). Lectins are sugar-binding proteins. Each lectin features a high specificity toward a particular carbohydrate structure; therefore even oligosaccharide with identical molecular compositions can be distinguished or separated. They are isolated from plants, microorganisms and animals. An example is Concanavalin A (ConA), isolated from Canavalia ensiformis beans (Goldstein et al., 1977). It is the multimeric structure of lectins which gives them their ability to form precipitates with glycoconjugates in a manner similar to antigen-antibody interactions (Kennedy et al., 1995). These conjugates can be fluorescently labeled, e.g. with fluorescein isothiocyanate (FITC). The use of these labeled lectins allows the microscopic in situ detection of EPS and their distribution in biofilms. This procedure has been demonstrated for multi-species biofilm by e.g. Lawrence et al. (1998); Neu and Lawrence (1999) or Takenaka et al. (2001).

Extracellular polysaccharides in the biofilm are stained with Lectin-Fluorescein isothiocyanate conjugate from Canavalia ensiformis (FITC-ConA) (Fluka, Buchs, Switzerland). The lectin is selected based on the specificity of the present protein for the EPS in the biofilm. ConA has a sugar specificity for D-glucose, D-mannose and sterically related sugars. In the biofilm, the two inoculating strains P. putida F1 and R. globerulus PWD1 should be present among others. P. putida as well as Rhodococcus strains are reported to produce EPS containing D-glucose and D-mannose (Iwabuchi et al., 2002; Kachlany et al., 2001; Read and Costerton, 1987). Circular pieces with a diameter of 20 mm are cut from a biofilm covered membrane that has just been removed from the reactor. The biofilm is
stained for 15 min at room temperature with 600 µl of a protein conjugate solution with a concentration of 100 µg/ml in PBS. All solutions are briefly spun down in a microcentrifuge before use, to remove any protein aggregates that may have formed. Only the supernatant is then added to the experiment.

For the acquisition of the emitted fluorescence the Leica TCS SP1 microscope is used again with 488 nm excitation and appropriate detector settings. For each scanned biofilm sample there are up to 20 circular ROIs analyzed, with an average total area of 9'800 µm². The intensity signals of all individual ROI are averaged, and the standard deviation is calculated for each focused level in the biofilm.

A calibration curve to obtain absolute EPS concentrations can not be done, not only because the composition of the EPS is unknown, but also due to the fact, that the absolute fluorescence intensity values are used. The light extinction is not circumvented by applying a relative measurement using two fluorescent stains, as it is the case for the live/dead analysis. The fluorescence intensity decreases with increasing measuring depth in the biofilm. Therefore a conclusion can only be dawn about the distribution of EPS in the biofilm for the first micrometers above the membrane.

### 3.9.3 Biofilm surface-profile / thickness

The biofilm thickness is measured with a laser distance sensor (LDS1-010, Raytec Systems AG, Chur, Switzerland). A modulated laser beam is generated by a semi-conductor laser diode. The laser beam is focused through a system of lenses on the target to be measured. The diffusive reflection of the laser beam is collected by a second system of lenses and measured by a position sensitive detector. The striking position of the beam of light on the detector is proportional to the distance between the target surface and the distance sensor.

The laser operates at a wavelength of 780 nm, the focus point of the laser on the target surface has a diameter of approximately 50 µm, and the accuracy of the available laser distance sensor is 25 µm. The maximum sampling frequency of the sensor is 500 kHz. The principle of using laser distance sensors for investigating the thickness of a biofilm is already
described by Vinage (2002). However, during the course of this study the described principle is applied to measure the biofilm thickness in situ, yet it is not only used to scan a line along the biofilm but rather a certain area of the biofilm, to obtain spatial information of its surface profile. The laser distance sensor is mounted on a motorized linear-track guide (LF5, Heeb Electro AG, Küsnacht, Switzerland) and a manual linear stage (426 equipped with SM-25, Newport, Darmstadt, Germany). These linear-track guides are installed on top of each other, and tilted against each other at an angle of 90°. The first guide is equipped with a step motor, allowing the movement of the sensor along the x-axis of the biofilm. The second small linear-track guide is used to move the laser-distance sensor in the y-direction. This combination allows scanning distinct lines along the x-coordinate of the biofilm. By displacing the sensor along the y-coordinate in steps of 500 μm and consecutively scanning lines along the x-axis, this set-up allows to draw a surface plot of the investigated area. A picture of the experimental set-up is shown in Figure 3.8.

The measurement through the cover glass of the narrow channel and the flowing aqueous phase requires a new calibration of the laser distance sensor. The light beams are refracted at the interfaces between the gas and the glass and between the glass and the water (see Figure 3.9). This lets the focused reflected light hit the position sensor at a different location leading to an incorrect distance measurement. A four point calibration is done by using distance measures (thicknesses 0.1, 0.25, 0.6 and 1 mm) placed within one flooded module onto the membrane and by correlating the measured voltage signal to the thickness of the measures. The distance sensor is moved along the x-axis with a velocity of 1 m/s and the distance measurements are averaged to a sampling frequency of 2 Hz, leading to a spatial resolution along a scanned line of 0.5 mm. Together with the above mentioned distance of 0.5 mm in the y-direction between the single lines, the analyzed grid consists of 25’000 data points of the investigated biofilm area.

3.9.4 Oxygen concentration profile in the biofilm

The concentration of dissolved oxygen in the biofilm is measured using a Clark-type oxygen microsensors (Ox10, Unisense A/S, Aarhus, Den-
Figure 3.8: The laser distance sensor is mounted on two linear track guides, which allows scanning a sector of the surface by (23 × 280 mm, marked with black lines in the picture).

The available sensors have tip diameters of only 10 μm, which allows a very high spatial resolution in the range of the diameter of the sensor tip. The oxygen sensor is mounted on a linear track guide driven by a step motor (Unisense, Aarhus, Denmark), allowing movement of the sensor in the z-direction. Step sizes and reading times are controlled by a computer. The sensor is mounted on a manually adjustable guide, which allows the movement of the sensor in the xy-plane. Therefore, dissolved oxygen profiles can be acquired at different positions of the biofilm. These track guides are fixed on module three (see Section 2). The above described pressure conditions within the four biofilm modules (see Section 2.2.5) allows module number three to be opened during operation of the reactor to acquire dissolved oxygen data along the height of the biofilm in situ. Oxygen concentration profiles are measured through the culture medium, the biofilm and the membrane into the silicone oil.
Figure 3.9: Using a laser distance sensor, the biofilm surface profile is analyzed in situ, through the glass plane and the culture medium.

3.9.5 Discharged biomass and elemental analysis

The discharged biomass and its elemental composition are important features that must be understood for two reasons: the carbon balance can only be closed if the amount of discharged biomass and its carbon content are available, and the amount and composition of the dry biomass is necessary for the calculation of the yield coefficients.

The discharged bacterial biomass is measured for each distinct steady-state operating condition by gravimetric analysis. An average of 200 ml of culture medium from the off flow is filtered through a nylon filter with a pore size of 0.45 μm (Schleicher & Schuell, Göttingen, Germany). For each steady-state condition, three consecutive samples are taken. The filters are dried in a vacuum oven at 100 Pa and 90 °C prior to and after the filtration. The difference in mass is weighed on a balance with an accuracy of ±1 · 10^-4 g. The elemental analysis is carried out at a specialized laboratory (Laboratory of Organic Chemistry, Service for microelemental analysis, ETH Zurich, Switzerland). The probes are investigated for their fraction of carbon, hydrogen and nitrogen. Since the fractions of the three elements do not equal one, the remaining mass
3.9. BIOLOGICAL INVESTIGATIONS

Figure 3.10: The set-up allows acquiring oxygen concentration profiles in the biofilm, through the membrane and into the absorbent.

fraction is assumed to be oxygen and ash Doran (1995). The discharged EPS is determined by total organic carbon (TOC) measurements (Dohrmann DC-190, Cincinatti, U.S.A.) of the filtrates from the gravimetrical analyses. The measuring equipment features an uncertainty of ±50 mg/l organic carbon.

3.9.6 Maximum growth rate

The maximum growth rate of the toluene degrading bacteria $\mu_{\text{max}}$ is determined using an indirect measurement, the oxygen consumption rate of the culture (Sapromat, H+P Labortechnik AG, Oberschleissheim, Germany). The oxygen consumption rate is detected by means of the oxygen transfer rate (OTR) to the bacterial culture. Basic to this measurement method is the assumption, that the OTR is proportional to the rate of
The initial biomass concentration in the culture liquid is denoted by $x_0$. Aerobic bacterial cultures consume oxygen during growth, maintenance and product formation and they produce carbon dioxide (CO$_2$). This depletion of oxygen causes a pressure drop in the closed flask holding the culture medium. The produced CO$_2$ would partly compensate for that pressure drop, therefore it is adsorbed by soda-lime. The pressure change in the flask is detected by a switch manometer, which induces the electrochemical production of O$_2$ by closing an electric circuit. The
cumulative amount of produced oxygen is registered to indicate the biological oxygen demand (BOD). The derivative of the BOD with respect to time, results in the OTR which is assumed to be proportional to the growth rate.

The natural logarithm taken from the OTR data are plotted versus the sampling time. The slope of the linear section of the curve specifies \( \mu_{\text{max}} \) (see Equation 3.9).

The experiments are carried out in 250 ml specially constructed Erlenmeyer flasks holding a small amount of soda-lime in the neck of the bottle. The samples are mixed using magnetic stirrers operating at 800 rpm. The reaction temperature is kept constant, by placing the flasks in a water bath at a temperature of 28\(^\circ\)C. The sample volume is 25 ml; it is inoculated under sterile conditions with 1.25 ml of a pre-culture. The measurements are done with toluene as the only carbon and energy source for the bacteria. The feed of toluene to the closed system is done analogous to the procedure described in Section 3.8.3.

The biofilm consortia as well as the single inoculated strains are tested for their \( \mu_{\text{max}} \). The biofilm is tested in two experiments, where the inoculum stems from a liquid pre culture, and from a freshly harvested biofilm sample. The pre-culture has been inoculated with a biofilm sample and has been growing in a shaking flask for 29 h in M9 at a temperature of 28\(^\circ\)C. The feed of toluene to the flask is done according to the above described procedure using a test tube (see Section 3.8.3). The biofilm sample is resuspended in M9 by vortexing for 5 min at maximum speed setting. The optical density (OD\(_{600}\)) is adjusted with M9 for both samples to a value of 2.1. The inocula for the \( \mu_{\text{max}} \)-investigations of \textit{P. putida} F1 as well as of \textit{R. globerulus} PWD1 stem from pre cultures grown on LB in test tubes at 28\(^\circ\)C. The cultures are spun down in 2 ml-Safe Lock vial (Eppendorf, Hamburg, Germany) at 16'100 g for 5 min (Centrifuge 5414D, Eppendorf, Hamburg, Germany). They are resuspended in minimal medium (M9) to a final OD\(_{600}\) of 6.5 and 2.4 for \textit{P. putida} and \textit{R. globerulus}, respectively.
Chapter 4

Results and discussion

4.1 Investigations on physical parameters

The investigations on the physical parameters in the present study cover
the mass transfer coefficients from the gas to the absorbent across the
membrane for toluene and oxygen and, the gas-absorbent as well as the
absorbent-nutrient medium partition coefficients.

4.1.1 Mass transfer across the membrane

The mass transfer across the membrane is analyzed from the gas phase
to the absorbent phase for the substrates toluene and oxygen.

4.1.1.1 Toluene

The overall mass transfer coefficient for toluene, between the bulk ab¬
sorbent and the bulk gas phase, based on the absorbent side, $k_{OV}^T$, is
$1.12 \cdot 10^{-6} \text{m/s}$ with a calculated total uncertainty of $\pm 3.4 \cdot 10^{-7} \text{m/s}$. It
is analyzed at a temperature of $20^\circ\text{C}$. This coefficient is a system spe¬
cific value which cannot be compared to other mass transfer coefficients.
reported in literature. The specificity is mainly due to the hydrodynamic boundary layer on the absorbent side. Therefore, the diffusion coefficient in the PDMS membrane, $D_{\text{memb}}^T$, which is a property independent of the specific configuration and therefore comparable, has to be calculated. Assuming that this diffusion coefficient in the membrane is constant (Cocchini et al., 2002; Sun and Chen, 1994), the calculation is done based on the resistance-in-series model approach (see Equation 3.2). The liquid film mass transfer coefficient in the silicone oil $\beta_{\text{ov}}^T$ is calculated to be $1.45 \cdot 10^{-6}$ m/s. This is determined by a $Sh$-correlation (Stephan, 1960) that uses the diffusion coefficient of toluene in silicone oil. Poddar et al. (1996) stated the diffusion coefficient of toluene in a similar silicone oil to be $8.5 \cdot 10^{-10}$ m$^2$/s. Based on these values $D_{\text{memb}}^T$ is estimated to be $(4.9 \pm 2.5) \cdot 10^{-10}$ m$^2$/s for a membrane thickness of 100 μm. This means that the membrane does not limit the mass transfer, and most of the resistance will be in the hydrodynamic boundary layer on the membrane. This finding is in agreement with literature data (Dossantos and Livingston, 1995; Ergas and McGrath, 1997; Livingston et al., 1998). In our case $(1/\beta_{\text{ov}}^T)$ exceeds $(\delta_{\text{memb}}/D_{\text{memb}}^T)$ by a factor of three.

The determined value for $D_{\text{memb}}^T$ is in agreement with comparable literature data, considering the different temperatures (see Table 4.1). The calculation of $D_{\text{memb}}^T$ based on $k_{\text{ov}}^T$ is carried out neglecting the mass-transfer boundary layer in the gas phase. This is a common assumption.

The rate of mass transport across the membrane often controls the performance of a membrane based biological process, as the operating conditions in the bio-compartment can usually be separately optimized (Livingston et al., 1998). Therefore, it is crucial to identify the main mass transfer resistance contributing to the overall resistance. Comparing the hydrodynamic mass-transfer resistance on the absorbent side, $1/\beta_{\text{ov}}^T$ with $1/k_{\text{ov}}^T$, it is found that the hydrodynamic boundary layer in the silicone oil is the main resistance to mass transport across the membrane, which separates the gas from the absorbent. This is in agreement with the results presented by others (e.g. Livingston et al., 1998). $\beta_{\text{ov}}^T$ is calculated according to the $Sh$-correlation presented in Section 3.4.3. It is important to reduce the thickness of the boundary layer, which is not only influenced by the flow regime but also by the membrane support. Thin
membranes are favorable since the membrane resistance $\delta_{\text{memb}} / D_{\text{memb}}^{S_i}$ is small. However, they often need a support structure for mechanical stability. Examples of such structures are coarse, porous membranes or metal braids. These supports prevent the fluid – at least on one side of the membrane – from freely flowing against the membrane surface, creating zones of standing fluids in the pores, increasing the thickness of the boundary layers, and therewith increasing the overall mass transfer resistance. Cocchini et al. (2002) found that the mass-transfer resistance increased by 40% across braided dense PDMS membranes compared to bare ones. This illustrates how advantageous the membrane type used in the present project is. The steel grid, applied for mechanical stability, is incorporated into the dense membrane material, where it does not influence the mass transfer. The bare surfaces allow free flow of absorbent along one side of the membrane and growth of biofilm on the other side. These flat membrane surfaces are also more resistant to biofouling (De Bo et al., 2002) as there is no porous support into which bacteria can grow or release EPS.

Table 4.1: Diffusion coefficients for toluene in PDMS membranes at different temperatures.

<table>
<thead>
<tr>
<th>$D_{\text{memb}}^T \cdot 10^{10}$ [m$^2$/s]</th>
<th>$T$ [°C]</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.15</td>
<td>25$^1$</td>
<td>Cocchini et al. (2002)</td>
</tr>
<tr>
<td>0.6 - 2.6</td>
<td>20</td>
<td>Sun and Chen (1994)</td>
</tr>
<tr>
<td>2.3</td>
<td>30</td>
<td>De Bo et al. (2002)</td>
</tr>
<tr>
<td>4.0 - 8.0</td>
<td>40</td>
<td>Favre et al. (2002)</td>
</tr>
<tr>
<td>4.9 ± 2.5</td>
<td>20</td>
<td>present work</td>
</tr>
</tbody>
</table>

$^1$ measured at room temperature
4.1.1.2 Oxygen

The diffusion coefficient of oxygen in the PDMS membrane is measured to be $(19.7 \pm 1.3) \cdot 10^{-9} \text{m}^2/\text{s}$. This value is calculated based on the relative analysis of the diffusion coefficient in water and the membrane (see Chapter 3.4.3.3). A plot of such a measurement is shown in Figure 4.1. The graph shows a typical oxygen concentration profile acquired through an agarose gel layer placed on top of the membrane, and through the membrane itself. As explained in Section 3.4.3.3 a strong reductant is placed on the opposite side of the membrane to keep up a constant flux of oxygen through the agarose/membrane films. The profile shows two consecutive straight lines and a rapid decrease of dissolved oxygen in the boundary layer of the reducing solution. According to Equation 3.6, based on the slopes of the linear curve fits, the diffusion coefficient in PDMS is 8.2 times higher than the one in the agarose gel, which is identical to the coefficient in water (Revsbech, 1989a) $(2.4 \cdot 10^{-9} \text{m}^2/\text{s at } 25^\circ \text{C})$ (Ramsing and Gundersen, 2004).

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**Figure 4.1:** This graph shows the two linear oxygen concentration gradients in an agarose gel and in the PDMS membrane. They are used to calculate the oxygen diffusion coefficient in the PDMS membrane, relative to the one in water.
The measurement is performed with swollen membranes between 80 to 150 µm thick (the thickness is determined by the oxygen profiles e.g. Figure 4.1). Toluene as well as the used silicone oil induce swelling of the membrane, which causes the membrane thickness to increase up to a factor of three. This swelling increases the oxygen diffusion coefficient. Compared to new, un-swollen membranes, with a thickness of only 55 µm, $D_{\text{memb}}^{O_2}$ is increased by 12% (measurements not shown).

When comparing the measured oxygen diffusion coefficients in PDMS, one finds that the few comparable literature data are all roughly ten to six fold smaller (see Table 4.2). There is no reasonable explanation, why the measured diffusion coefficient differs so much from the ones reported in literature.

<table>
<thead>
<tr>
<th>$D_{\text{memb}}^{O_2} \cdot 10^9 \left[ \frac{m^2}{s} \right]$</th>
<th>$T \left[ ^\circ \text{C} \right]$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.6$^a$ ± 1.1</td>
<td>30</td>
<td>De Bo et al. (2002, 2003)</td>
</tr>
<tr>
<td>3.4 ± 0.1</td>
<td>35</td>
<td>Merkel et al. (2000)</td>
</tr>
<tr>
<td>19.7 ± 1.3</td>
<td>25</td>
<td>present study</td>
</tr>
</tbody>
</table>

$^a$ The value is calculated based on permeability and solubility data.

Based on the measured oxygen diffusion coefficient, the overall mass transfer coefficient between gas and absorbent, $k_{ov}^{aO_2}$, can be estimated. This coefficient is used later in the model (Chapter 5). The calculation of $\beta^{aO_2}$ is done with the height of the free channels in the static mixers of 5 mm. The liquid velocity of $1.94 \cdot 10^{-3} \text{ m/s}$ is calculated from the flow rate of absorbent through the bioreactor modules at standard conditions ($20 \frac{1}{h}$). $\beta^{aO_2}$ is estimated to be $2.2 \cdot 10^{-5} \text{ m/s}$. The overall mass transfer
coefficient is calculated according to the resistance-in-series model, using a membrane thickness of 100 μm and the calculated \( D_{\text{memb}}^{O_2} \). \( k_{\text{ov}}^{aO_2} \) is calculated to be \( (1.87 \cdot 10^{-5} \pm 1.4 \cdot 10^{-6}) \text{ m/s} \). The uncertainty is calculated with an assumed variation of the membrane thickness between 50 and 150 μm and with the calculated error of the diffusion coefficient. The resistance of the hydrodynamic boundary layer \( (1/\beta^{aO_2}) \) exceeds the resistance of the membrane \( (\delta_{\text{memb}}/D_{\text{memb}}^{O_2}) \) by factors between 6 and 18 for the different membrane thicknesses.

The high overall mass transfer coefficient for oxygen and toluene in the thin PDMS membrane is a big advantage for application in membrane bioreactors. Aerobic biofilms that grow on standard membranes often encounter insufficient amounts of oxygen for optimal and complete metabolism of pollutants (De Bo et al., 2003).

4.1.2 Partition coefficients

The partition coefficients for substances that are widely used, such as toluene and oxygen, are commonly known for air-water systems (e.g. Sander, 1999). However, partition coefficients for systems including silicone oil, which is not a defined polymer with a known stoichiometric formula and features only an average molecular weight, require experimental analyses.

4.1.2.1 Gas-absorbent

**Partition coefficient for toluene** The gas-absorbent partition coefficient, \( H_{\text{gaT}} = c_{\text{gT}}/c_{\text{aT}} \), increases with increasing temperature and decreases with higher toluene concentrations (see Figure 4.2). These values, measured in independent experiments, can also be well compared to the gas-absorbent partition coefficient for toluene in the course of analyzing the membrane bioreactor at steady-state. In Figure 4.6 the toluene silicone oil concentration is plotted versus the toluene outlet gas concentration. The inverse value of the slope of the regression line is \( H_{\text{gaT}} \), it is \( 0.932 \cdot 10^{-3} \pm 1.4 \cdot 10^{-5} \).
The measured coefficients are also comparable to partition coefficients reported by Poddar and Sirkar (1996). The values in that study also increased in correlation with temperature; however, they are lower than the measured data by a factor of approximately 0.5. The silicone oil used was not exactly the same and the concentration level at which the measurements were carried out are not explicitly declared.

**Partition coefficient for oxygen**  The gas-absorbent partition coefficient for oxygen, \( H^\text{gas}_\text{O}_2 = c^\text{g}_\text{O}_2 / c^\text{a}_\text{O}_2 \), is measured to be 5, with an uncertainty of ±15% (an estimate based on the thermodynamical equilibrium law, using known partition coefficients. See Section 4.1.2.2). There are no comparable literature data available with a similar silicone oil. However, De Bo et al. (2002) stated an oxygen partition coefficient for PDMS membranes (dense PDMS is structurally identical with silicone oil, but features a much higher molecular weight) of \( 2.2 (±0.9) \), which is in the same range.
4.1.2.2 Water-absorbent

Partition coefficient for toluene  The measured data of the water-absorbent partition coefficients, \( H_{mT} = \frac{c^mT}{c^aT} \), are shown in Figure 4.3. The error bars in the graph stand for the standard deviation of the three individual, averaged measurements. It is obvious that the determined partition coefficients for toluene in the considered concentration and temperature range, are identical with an average value of \( 3.6 \cdot 10^{-3} \pm 7 \cdot 10^{-4} \). There are no available literature data to confirm the correctness of the result, however, checking for the appropriate range can be done by applying the thermodynamic equilibrium law. This law states, that if phase A is in equilibrium with phase B, and phase B is in equilibrium with phase C, then phases A and C must be in equilibrium. Therefore, the gas must be in equilibrium with the water. The partition coefficient describing this ratio \( H^{gmT} \), defined as \( \frac{c^gT}{c^mT} \), can be found in literature (e.g. Hoff et al., 1993; Mackay and Shiu, 1981; Nielsen et al., 1994; Robbins et al., 1993; Wasik and Tsung, 1970). The average of these selected values, at a temperature of 25 °C, is 0.26 ± 0.03 (the error margin denotes the deviation of the measurements, not uncertainties of

![Figure 4.3: The dimensionless partition coefficient for toluene between the aqueous phase and the absorbent as a function of temperature.](image-url)
the individual analyses).

\[ H_{gmT}^{T} = \frac{c_{gT}}{c_{mT}} = H_{gaT}^{T} = \frac{c_{gT}}{c_{aT}} \]

Following Equation 4.1 the partition coefficients \( H_{gaT}^{T} \) and \( H_{maT}^{T} \) can be compared with \( H_{gmT}^{T} \). The value for \( H_{gaT}^{T} \) is interpolated for a temperature of 25 °C, it varies between 0.82 \( \cdot 10^{-3} \) and 1.06 \( \cdot 10^{-3} \) for the analyzed concentration range. The resulting \( H_{gmT}^{T} \) range falls between 0.23 and 0.29, in perfect agreement with the air-water partition coefficient for toluene found in literature. It can be concluded therefore, that the obtained partition coefficients for toluene and the three phases are reasonable.

**Partition coefficient for oxygen** The dimensionless water-silicone oil partition coefficient for oxygen, \( H^{maO_2} = \frac{c^{mO_2}}{c^{aO_2}} \), is measured to be 0.12, with an estimated error ratio of ±15%. There are no literature data available for comparison, but it can be checked by analogy with toluene (see Equation 4.1). The averaged literature gas-water partition coefficient for oxygen \( H^{gmO_2} \), defined as \( \frac{c^{gO_2}}{c^{mO_2}} \), is 32.3 ± 1.4 (e.g. Carpenter, 1966; Dean, 1992; Kavanaugh and Trussell, 1980; Lide, 2004; Wilhelm et al., 1977) (the error margin refers to the deviation between the single measurements). The coefficient \( H^{gmO_2} \), calculated based on \( H^{gaO_2} \) and \( H^{amO_2} \), is 41.6±8.8. This calculation confirms that the partition coefficients for oxygen are more difficult to be precisely determined, however considering the stated uncertainty range they must be within the proper magnitude.

### 4.2 Reactor performance under steady-state conditions

The reactor is operated without major problems for 162 days. A visible biofilm developed on the membrane of the bioreactor after a few days, as illustrated in Figure 4.4. The reactor is run for 60 days at operating condition one to achieve a stable toluene degrading bioreactor. Starting
Figure 4.4: A picture showing the biofilm, developed on the membrane 24 days after inoculation. The static mixers used as support bearing for the membrane are mirrored in the biofilm surface, due to substrate limitation at the supporting edges.

from this working point eight further steady-state operating conditions are run and analyzed. These conditions are run for three distinct gas flow rates and feature a wide range of inlet toluene concentrations. Figure 4.5 shows the resulting outlet gas concentrations for these nine steady state operating conditions. Toluene is readily degraded by the bacteria. The error bars represent the total uncertainties.

In this operating mode the system is allowed to reach steady-state for nine different inlet conditions. This is shown by plotting the toluene silicone oil concentration versus the outlet gas concentrations for all steady-state operating conditions (see Figure 4.6). The data points lie on a straight line. This signifies that the gas is in equilibrium with the absorbent for all operating conditions independently of the gas flow rate or the gas residence time in the absorption module. The gas-absorbent contact time is sufficiently long, what implies that the absorbtion mod-
Figure 4.5: The toluene outlet gas concentration is shown as a function of the inlet gas concentration and the gas flow rate for the nine steady-state operating conditions. The dotted line represents the same inlet and outlet concentration.

The toluene is built long enough to allow establishing equilibrium also for the larger gas flow rates.

Figures 4.5 and 4.6 show both the measuring mistake of one data point. At the operating condition nine – $c_0^{gT} = 0.625 g/m^3$ and $V^g = 4 l/min$ – the system does not reach equilibrium when the data is acquired. For this data point the toluene outlet gas concentration is higher than the inlet gas concentration (see Figure 4.5) and the toluene silicone oil concentration as a function of the outlet gas concentration does not lie on the same equilibrium line as the remaining eight data points (see Figure 4.6). In spite of this mistake this erroneous measurement is analyzed together with the ones of the remaining steady-state operating conditions.
Figure 4.6: The toluene concentration in the silicone oil is in equilibrium with the outlet gas concentration for the nine steady-state operating conditions. The dotted line represents the linear regression of the data points.

4.2.1 Degradation performance

4.2.1.1 Surface elimination capacity

The surface elimination capacity of toluene is defined as the mass of toluene removed from the gas stream per time, related to the membrane area. The surface elimination capacity $SEC^{bT}$ is based on the biofilm area $A_{memb}^{b}$:

$$SEC^{bT} = \frac{1}{A_{memb}^{b}} \left( \left( c_{0}^{bT} - c_{out}^{g} \right) \dot{V}_{g} - c_{mT}^{m} \dot{V}_{m} ^{m} \right)$$

$$\approx \frac{\dot{V}_{g}}{A_{memb}^{b}} \left( c_{0}^{bT} - c_{out}^{gT} \right). \quad (4.2)$$

The toluene gas concentration at the inlet and at the outlet of the reactor are denoted $c_{0}^{gT}$ and $c_{out}^{gT}$, respectively. The toluene concentration in the mineral medium is called $c_{mT}^{m}$ and $\dot{V}_{m}$ denotes the flow rate of the bleed stream. Usually the second term in the equation, describing the mass of
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aqueous VOC transported out of the reactor by the bleed stream of the mineral medium, is neglected (e.g. Vinage, 2002). This assumption also holds for poorly water soluble compounds and low surface loads. The calculations of the $SEC^{bT}$, based on the experimental data, also have to be done without the liquid concentration, since – as will be seen later in Section 4.2.2.3 – the toluene concentration in the culture medium is below the detection limit. However, the analyses of the $SEC^{bT}$ for periods of very high loads at the reactor inlet require the inclusion of the toluene concentration in the culture medium, since considerable amounts of toluene dissolved in the aqueous phase are discharged. Very high inlet gas loads are only modeled to determine the maximum elimination capacity of the set-up (see Section 5.5.3).

In the following graphs, data are plotted against the surface load $\dot{m}_{\text{memb}}^{aT}$, defined as the mass flux of toluene based on the membrane area provided for absorption (see Equation 4.3).

$$\dot{m}_{\text{memb}}^{aT} = \frac{\dot{M}^T}{A_{\text{memb}}}$$  (4.3)

The surface elimination capacity is based on the membrane area available for biofilm growth in the bioreactor modules; while the surface load is based on the membrane area in the absorption module. The biofilm area amounts to 0.27 m$^2$, whereas the absorption area is only 0.18 m$^2$.

The surface elimination capacity increases with increasing surface load (see Figure 4.7). The large calculated uncertainties as well as the inconsistent order of the elimination capacities regarding the gas flow rate, make it impossible to draw a conclusion regarding the influence of the residence time in the absorber on the reactor performance.

The total uncertainties include the random errors of the measured data as well as the systematic errors of the measuring devices, stated by the manufacturers. The ordinate-error bars represent the total uncertainty calculated of the systematic and the random errors. Systematic errors include errors of the gas mass flow controller $\delta \dot{V}_{\text{sys}}^g$, the toluene mass flow controller $\delta \dot{M}_{\text{sys}}^T$ and the FID, used to measure the toluene gas concentration at the outlet, $\delta c_{\text{sys}}^T$. Only the random error of the toluene gas concentration is taken into account $\delta c_{\text{ran}}^T$, which is the standard deviation.
The surface elimination capacity, based on the biofilm area, is plotted against the toluene surface load, which is calculated using the membrane area provided for absorption. The error bars mark the total calculated uncertainties.

of the measurements taken during the period of the individual steady-state working points. The inlet concentration is calculated using data taken from the two flow controllers. The abscissa-error is only a function of $\delta \hat{M}_{\text{sys}}^T$. The measured data points for a certain surface load are all within the ordinate-uncertainty ranges of each other. Additionally the succession of the surface elimination capacities, as a function of the different gas flow rates, is not the same for the three distinct surface loads. A z-test applied on each of the three data points measured for a certain surface load confirms what is presumed from the plotted error bars; based on a 5% significance level, the results from the 3 measurements are statistically indistinguishable.

Based on measurement results, it has to be concluded that the surface elimination capacity is independent of the gas flow rate or rather of the residence time of the gas in the absorber. The surface elimination capacity increases with increasing surface load along a non-linear pattern. The curve seems to level off at a distinct surface load. The increasing behavior is an indication for a mass transfer limited regime. The bigger the
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concentration gradient between the gas and the aqueous medium gets –
the driving force for the absorption and the transport of the toluene to
reach the bacteria – the more substrate is degraded.

A reaction limited regime is characterized by a constant elimination ca-
pacity. An increase in substrate concentration does not lead to a higher
degradation capacity. The bacterial consortium works at the strain-
specific maximum rate. From Figure 4.7 it cannot be concluded whether
the system can already be considered to be in the reaction limited regime
at a surface load of 3.29 g/m²·h. This conclusion would require further ex-
perimental data obtained at higher surface loads or a model describing
the existing system (see Chapter 5.5).

The measured surface elimination capacities are compared to data re-
ported on toluene degrading membrane bioreactors (see Figure 4.8). The

toluene inlet gas concentrations, the gas flow rates as well as the volumes
of the reactors varied widely. The surface load takes the gas concentra-
tion and the gas flow rate into account. However, the ratio of the gas
volume flow to the reactor volume is not considered. Therefore to com-
pare the data, the attempt is made at classifying the information based
on the residence time $\tau$.

![Figure 4.8](image)

**Figure 4.8**: Comparable literature data on the performance of toluene de-
grading membrane bioreactors.
Figure 4.8 shows that the literature data are in agreement with the data obtained in this study. This holds true except for one data point reported by Hartmans (1992) on the performance of one of the first toluene degrading membrane bioreactors. The residence time seems to be of minor importance for the considered range. For very low surface loads the residence times are small and generally seem to increase concurrently with the loads.

### 4.2.1.2 Removal efficiency

The reactor performance can also be described in terms of its removal efficiency $RE$. It is defined as the fraction of the mass of VOCs fed to the reactor, which is removed from the environment, expressed in percent:

$$RE = \frac{\dot{V}g \left( c_{0}^{gT} - c_{out}^{gT} \right) - \dot{V}m c_{mT}}{\dot{V}g c_{0}^{gT}} 100$$

The removal efficiency is often only calculated as the fraction of the supplied mass to the reactor, removed from the waste gas stream, neglecting the mass carried out by the bleed stream of the culture medium. Again, this assumption only holds for poorly water soluble compounds and for low inlet concentrations. The experimentally determined removal efficiency is calculated only by means of the gas concentration, since the toluene concentration in the culture medium is below the detection limit of the measurement device (< 5 mg/l).

The removal efficiency decreases with increasing surface load (see Figure 4.9). There is no perceivable trend perceptible for changing gas flow rates (e.g. one would expect that for higher flow rates the removal efficiency would continuously decrease). The plot of the removal efficiencies confirms the results obtained in the analysis of the surface elimination capacity, the gas flow rate is of no influence on the removal efficiency for a distinct surface load. The conclusion is, that the residence time in the absorber is also sufficiently long to establish equilibrium for high gas flow rates of $4 l/min$. 

\[4.4\]
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Figure 4.9: The removal efficiency as a function of the surface load. The influence of the gas flow rate is of no significance. The error bars represent the total uncertainties.

4.2.2 Substrate and product concentrations in the gas, absorbent and culture medium

In the following subchapters the concentrations of toluene and oxygen in the gas, absorbent and aqueous phase are presented and discussed. These concentrations do not allow characterization of the degradation performance of the system. However, they lead to conclusions with respect to possible, biodegradation limiting concentrations of the substrates in question.

4.2.2.1 Gas

The concentration of toluene in the gas phase at the outlet of the reactor is shown as a function of the surface load applied (see Figure 4.10). The increase of toluene concentrations for the three distinct gas flow rates seems to increase linearly with increasing surface loads. However, a straight line would indicate, that a constant fraction of the fed toluene flux is removed from the waste gas stream, which is not the case. In the
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Figure 4.10: The toluene gas concentration at the outlet of the reactor as a function of the toluene surface load and gas flow rate. The error bars represent the total uncertainties.

course of the analysis of the removal efficiency, the fraction of removed VOC is lowered for higher loading rates. Therefore, the toluene outlet gas concentrations must increase concurrent with surface load. The data point measured at a gas flow rate of 2 L/min and at a surface load of 3.29 g/m²/h should be higher (see Section 4.2.3). The data points for the distinct gas flow rates are stacked in the order of decreasing flow rates, because for a constant mass flow rate of toluene, $\dot{M}^T$, the inlet toluene gas concentration has to change. It is highest for the lowest gas flow rate. The ordinate error bars represent the total uncertainties for the measured gas concentration at the reactor outlet.

The oxygen concentration in the waste gas is not measured. However, a mass balance can show that the oxygen concentration in the air changes between the inlet and the outlet of the reactor by a maximum 2% (for the steady-state working point number one). The oxygen concentration in the gas is therefore of minor interest.

The concentration of CO₂ in the gas phase at the outlet of the reactor is virtually constant for a distinct gas flow rate in the considered surface load range (see Figure 4.11). All CO₂ present in the gas phase – except
the small amount naturally present in the atmosphere – is produced by the bacteria in the course of degradation of toluene and must diffuse back from the biofilm through the absorbent and desorb into the gas phase. The CO$_2$ concentration in the gas is higher for lower gas flow rates. However, the concentration at a gas flow rate of 1 l/min is not four times higher than the one at 4 l/min, leading the conclusion that for a smaller toluene inlet concentration, the substrate uptake rate for maintenance is more pronounced. The reduced substrate availability for the bacteria leads to an increased CO$_2$ fraction of the reaction products (see also Section 4.2.3).

4.2.2.2 Absorbent

The measured toluene concentration in the silicone oil is presented as a function of the applied surface load (see Figure 4.12). The concentration in the silicone oil increases with increasing surface load and with decreasing gas flow rates. The toluene concentration in the gas increases, due to lower gas flow rates while the concentration in the absorbent increases.
Figure 4.12: The toluene concentration in the silicone oil as a function of the surface load.

The pattern of the data points is analogous to the one of the toluene concentration in the gas phase at the reactor outlet. This is expected, if the gas and the absorbent are in equilibrium. This indicates that even for the maximum gas flow rate, the absorber module is built sufficiently long to reach equilibrium.

The toluene gas concentrations are considerably small with values of 0.6 to 10 g/m$^3$, while the corresponding concentrations in the silicone oil are approximately 1,000 times higher, due to the favorable gas-absorbent partition coefficient for toluene. This high buffer capacity for VOCs, such as toluene, makes silicone oil a preferred absorbent.

The small ordinate error bars in the graph represent the uncertainty of the HPLC analyses. The total uncertainty of the measured data is most likely underestimated. The error, introduced for example, by the suboptimal mixing of the total absorbent volume in the reactor, is not known.

The oxygen concentration in the absorbent is not reliably determined. A Clark type oxygen probe, which is designed for aqueous media, is used for measurements in the silicone oil. The determined concentration data vary between 60% and 70% of the value representing saturation with
air. This seems reasonable, however there is no clear trend perceivable, e.g. an expected slight decrease with increasing surface load. It has to be concluded from these measurements that oxygen probes designed for fermenters do not function properly in hydrophobic environments.

4.2.2.3 Culture medium

The toluene concentration in the nutrient medium is measured off-line by means of GC analysis. The detection limit for the measuring device is in the range of $5\text{mg/l}$. The obtained data points all lie within this range. It is not possible to indicate a trend, e.g. a suspected increase for higher surface loads at low gas flow rates.

Figure 4.13 shows the oxygen concentration in the culture medium as a function of the surface load. This plot demonstrates that only the oxygen concentration in the culture medium is considerably different from zero for low surface loads together with high gas flow rates. These are the steady-state working conditions at $1.64\text{s/m}^2\text{h}$ with the maximum gas flow rate of $4\text{l/min}$, and the working conditions at the lowest adjusted
surface load of 0.82 s/m²h, featuring gas flow rates of 4 l/min and 2 l/min, respectively. These seem to be the steady-state working conditions of low toluene but high oxygen feed rates. However, as discussed in Section 4.2.2.1, the oxygen concentration in the off-gas is subject to only minor changes, therefore it is reasonable to assume toluene inlet gas concentrations are the major cause for higher dissolved oxygen levels at the above stated steady-state working points. The toluene inlet gas concentrations are low, with values between 0.625 s/m³ and 1.25 s/m³, causing decreases in the concentration gradients and the mass transfer rates for toluene, while the oxygen rates remain constant.

This observation implies, that degradation in the culture medium is only under three steady-state working conditions definitely not oxygen limited. The considerable amount of dissolved oxygen in these cases also signifies that the oxygen in the biofilm cannot be entirely depleted; all oxygen present in the culture medium must have been transported through the biofilm, because the culture medium is closed against the environment. For all other working points it is not possible to state whether at all, or to what extent, the bacterial degradation suffers from oxygen limitation.

The total CO₂ concentration in the culture medium, the sum of CO₂(aq) and HCO₃⁻, is shown in Figure 4.14. The cumulated CO₂ concentrations for the distinct nine steady state working points are statistically indistinguishable. The error bars denote the total calculated uncertainty of the CO₂ measurements in the medium. The values are considerable compared to the oxygen and the toluene concentrations in the medium.

The pH is measured on-line in the mineral medium. It is constant for all analyzed conditions, the average value amounts to 6.93±0.02. The mineral medium is buffered by means of Na₂HPO₄. The buffering capacity is sufficiently large to balance the pH drop introduced by the formation of HCl and H₂CO₃.

### 4.2.3 Carbon balance

A carbon balance over a biological waste gas treatment reactor is a good check of the quality of the measurements carried out during operation
There are three carbon flows crossing the boundary of the balance region set to enclose the whole set-up. One flow enters the system, two leave it. The only carbon flow entering the balance region consists of gaseous toluene and of 300 ppm carbon dioxide naturally occurring in the environment. A gaseous and an aqueous stream leave the system. The gas contains carbon in the form of toluene and of CO₂. It is assumed that no volatile degradation products are formed by the bacteria. CO₂ constitutes the inorganic carbon fraction in the outflow of the culture medium, present in water as aqueous CO₂ and at the pH of 6.9 – as hydrogen carbonate. The organic fraction is made of dissolved toluene, biomass and intermediates. The biomass can be subdivided in bacteria and in exopolymeric substances.

Summing up these inflowing and outflowing carbon streams results in the carbon balance for the nine steady-state working points (see Figure 4.15). The flow rates are represented as moles of carbon per hour. The total inflowing carbon stream is – by looking at Figure 4.15 – in very good agreement with the outflowing stream, except for two steady-state working conditions. These two conditions feature a toluene mass flow

**Figure 4.14:** The total CO₂ concentration in the culture medium is shown as a function of the toluene surface load.
Figure 4.15: The molar carbon flow rates into and out of the reactor are plotted versus the steady-state working conditions. They are characterized by the gas flow rate (upper line of the abscissa label) and the mass flow rate of toluene (lower line).

The graph shows the three distinct mass flow plateaus, which are reached in the course of the steady-state experiments. The calculated molar carbon flow rate out of the reactor is constantly lower than the inlet flow rate. One explanation is, that the inaccuracies of the mass flow controllers in the loading unit – both used at the lower end of the adjustable range – add up to produce a constantly higher toluene flow rate. This explanation is supported by concentration measurements of the inlet gas before the reactor has been started. It is also supported by the rather large systematic error (±1% of the maximum value) stated by the manufacturer of the loading unit. On the other hand it is also possible that other unknown products are formed, which are not detected and
therefore reduce the molar carbon flow rate out of the reactor.

Once it is shown that the carbon balance is "closed" – the same amount of carbon is entering and leaving the balance region – the fraction of toluene leaving the reactor untreated, must be determined. In the discussion of removal efficiency, this may amount to a significant percentage in lab scale reactors. Figure 4.16 shows the fraction of the total carbon leaving the reactor, which makes up for toluene in the gas and aqueous phases. The measurements of toluene in these two phases, with the corresponding uncertainties, are discussed in chapters 4.2.2.1 and 4.2.2.3.

The fraction of untreated toluene increases with increasing toluene mass flow rate. The percentage of discharged toluene that is dissolved in the culture medium is insignificant, due to the poor water solubility and the virtually complete degradation of the dissolved toluene.

The degradation products consist of carbon dioxide (CO₂) and biomass. The composition of the carbon at the outlet stemming from the degraded toluene is shown in Figure 4.17. This flow is composed of inorganic CO₂ and of organic biomass. CO₂ leaves the reactor in three states: gaseous, aqueous and as hydrogen carbonate. The ratios between these three states are similar for all steady-state working points. The CO₂

![Figure 4.16: The fraction of untreated toluene leaving the reactor is shown for the applied steady-state working points.](image)
contributes the biggest fraction of the different phases, in general around 80%. Gaseous CO₂ constitutes more than three quarters of this amount, implying that this part of the produced CO₂ has to diffuse back — unlike toluene and oxygen — through the biofilm into the absorbent and from there, it has to desorb into the waste gas. The remaining quarter is composed of only 3% aqueous CO₂, explicitly measured by a CO₂-probe, the rest of the dissolved CO₂ is carried out in form of carbonate. This amount is calculated based on the CO₂(aq) measurement together with the measured pH value of 6.9 for all working conditions, and the temperature determined in the culture medium of 28.3°C, using the Henderson-Hasselbach Equation (see Chapter 3.5.2.5).

The analysis of the biomass fraction in the carbon balance is based on the assumption that there is no net biofilm accumulation or removal. Biomass is eroded at the same rate as it grows. This assumption is supported by the biofilm thickness measurements, which turned out to remain virtually constant during the steady-state experiments (see Section 4.4.2). The biomass fraction is composed of bacteria and EPS. Approximately 20% of the organic carbon in the aqueous phase is made up by the bacteria. The total bacterial mass is determined by filtering off
a certain amount of the culture liquid, drying the remains and analyzing them gravimetrically. A filter with a pore size of 0.45 μm is used. For the extraction of EPS from biofilms generally filters with a pore size of 0.45 μm are used (Nielsen and Jahn, 1999). Therefore, it can be assumed that the main part of the EPS passes through the filter and the bacteria will be held back. The EPS content in the filtrate is analyzed by TOC measurements. However, the TOC content in the filtrate is small and lies in the range of the detection limit of the TOC measuring device. A constant value of 50 mg/l is assumed for all working points.

4.3 Reactor performance under dynamic conditions

The buffering capacity of the novel set-up is tested by means of feeding fluctuating loads to the reactor and by analyzing the responding toluene concentration in the gas at the reactor outlet. The fluctuating loads are simulated by varying the mass flow rate of toluene for different, distinct gas flow rates. Figure 4.18 shows a typical plot of fluctuating inlet gas concentrations for a gas flow rate of 1 l/min with the corresponding outlet gas concentration. The solid line represents the inlet gas concentration, the markers indicate the measured outlet toluene concentration. For the data point at 25 h, the systematic error of the toluene outlet gas concentration is shown, it is insignificantly small. Starting from the steady-state working point number four with a toluene concentration of 5 g/m³, a possible working day in a VOCs emitting industry is simulated. At time A the mass flow of toluene is shut down, simulating the begin of a night. The period without toluene feed lasts for 18 h, until at time B the next working day starts. This day is characterized by high, peaking loads and a period of no toluene feed, e.g. lunch time.

The concentration of the outlet stream is virtually constant. This pattern confirms that buffering of VOCs is achieved with this system. Although the inlet gas stream shows high load fluctuations, the loading of the outlet stream is constant. Assuming that the gas and the absorbent are in equilibrium – as demonstrated before, in figures 4.10 and 4.12 – this means that a large amount of toluene is stored in the absorbent. In
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Figure 4.18: The course of fluctuating inlet gas concentrations during a possible working day in a VOCs emitting industry is shown. In the time span A-B there is no toluene fed to the reactor. The outlet gas concentration shows only minor changes. The graph shows an experiment run at a gas flow rate of 1 litre/min.

The period A-B without toluene feed, toluene desorbs from the silicone oil into the gas phase and leaves the reactor.

The main advantage of this buffering is to enable a constant feed rate of VOCs to the bacteria in the biofilm, in spite of periods of no toluene or very high loads present in the inlet gas. As a consequence of the absorption, however, toluene will leave the reactor even when there is no toluene in the in-flow. This is due to the fact that the off-gas is in equilibrium with the scrubbing liquid. In case of an achieved absorption equilibrium, the concentration in the off-gas cannot be exactly constant, but must follow the inlet gas concentration (see Figure 4.19). The same plot as Figure 4.18 is redrawn with a higher resolution for the outlet gas concentration. The curve clearly follows the pattern of the inlet gas concentration. Each concentration change of the inlet gas is mirrored in the outlet gas concentration. At higher resolution it can also be seen, that the concentration of toluene in the absorbent diminishes during the experiment. This is due to biological degradation since an integrative
4.4 Biological investigations

4.4.1 O₂-concentration profiles in the biofilm

The concentration of dissolved oxygen in the biofilm is measured along the z-axis, using an oxygen microsensor. Oxygen concentration profiles are acquired at different positions on the biofilm (in the reactor opening in bio-module three, see Figure 3.11). An oxygen concentration profile is measured through the culture medium, the biofilm, the membrane and into the silicone oil. An averaged example of three dissolved oxygen concentration profiles, acquired in the course of the steady-state working point number one, 40 days after reactor start-up, is shown in Figure 4.20. There is no dissolved oxygen in the nutrient medium. This result is
Figure 4.20: An averaged dissolved oxygen concentration profile, acquired along the $z$-axis in the biofilm of the experimental facility.

in agreement with the on-line measurement of the oxygen concentration in the culture medium (see Section 4.2.2.3). The boundary between the nutrient medium and the biofilm can not be readily assigned based on the oxygen profile; therefore, the drawn border should not be considered as the exact position. Biofilm thickness measurements show that the value for this operating point, is in the range of 400 $\mu$m (see Figure 4.22, 46 d). The course of the oxygen concentration shows that no oxygen is detectable in a large portion of the biofilm. The oxygen concentration increases rapidly in the last approximately 80 $\mu$m of the biofilm towards the membrane. All oxygen delivered to the biofilm must pass through the membrane, therefore, the highest oxygen concentration in the aqueous phase, must be located on the membrane. Remarkably, however, all the oxygen is depleted in a short distance.

The oxygen concentration in the dense PDMS membrane increases linearly with penetration depth. This is a clear indication for a diffusion process in the dense membrane matrix.

The concentration profile below the membrane, in the silicone oil, does not show an abrupt change in its course, as it is the case at the membrane biofilm interface. The oxygen concentration increases over a distance of
approximately 100 μm to reach a constant value in the bulk phase of the absorbent. This detected value is in agreement with the on-line measured value of dissolved oxygen in the silicone oil of (71.9 ± 1.1) %. This curve progression suggests a rather thick boundary layer on the membrane in the absorbent phase, in spite of the use of static mixers.

The thin biofilm layer, in which oxygen is present, requires a higher resolution of the oxygen concentration profiles at this depth range. Figure 4.21 shows the three independent oxygen profiles acquired during steady-state of operating point number one, in the range of 200 μm distance from the membrane. The curve progression in the biofilm shows

![Figure 4.21](image)

**Figure 4.21:** Three independent oxygen concentration profiles, acquired in the base layer of the biofilm, closest to the membrane. The curves show inflection points in the course of the profiles.

that only the base layer is definitely not oxygen limited. This finding confirms the assumption that the most active area of the biofilm is its base. The highest toluene degradation rate would therefore not occur at the biofilm surface but close to the membrane. Live/dead analysis confirmed this assumption (see Section 4.4.7). Results of these measurements, detailing the fraction of living to dead bacteria, show a decrease to zero over the first 90 μm of the biofilm, next to the membrane. Therefore, it can be concluded that the most active toluene degrading layer is
at the base of the biofilm. This is in contrast to natural biofilms, which obtain oxygen through the aqueous phase flowing along them.

The course of the three higher resolved curves shows a second surprising result of the oxygen microsensor analyses in the biofilm. The curves change their curvature from convex to concave, with an inflection point at approximately 60 μm distance from the membrane. The biofilm grows directly on the membrane, therefore, it would be expected that the oxygen concentration decreases exponentially, starting on the membrane. This curvature leads to the conclusion that the diffusion flux into the biofilm exceeds the consumption rate in the first, approximately 60 μm of the biofilm, next to the membrane. This phenomenon, as well as oxygen concentration profiles for aerobic membrane bioreactors, have not been reported in literature. Possible explanations for this curve progression are, (i) that in this oxygen and toluene buffering membrane bioreactor a higher oxygen flux to the biofilm occurs (exceeding the consumption rate) compared to biofilm reactors, where the oxygen is fed through the top layer; and (ii) an additional layer is located directly on the membrane, protecting the cells from growth inhibiting or toxic toluene concentrations, and at the same time also increasing the mass transfer resistance of oxygen.

A calculation of the oxygen flux to the membrane is done, based on the slope of the concentration gradient in the membrane, on the determined oxygen diffusion coefficient in the membrane (see Section 4.1.1.2), as well as under the assumption that the water-silicone oil partition coefficient is in the same range as that for water-PDMS (see Section 4.1.2.2). The oxygen flux to the biofilm is estimated at a value of $(1.22 \pm 0.07) \times 10^{-3} \, \text{s/m}^2\text{s}$. This flux is e.g. five times higher than the oxygen transport rates reported by Debeer et al. (1994) for a biofilm fed with oxygen from the overflowing aqueous medium. The correctness of the calculated flux can be checked by simply calculating the required oxygen for the degradation of the toluene fed to the reactor, using the stoichiometrical coefficients of toluene and oxygen derived in Equation 4.15. The mass flux of toluene, during steady-state conditions of working point number one, amounts to $1.76 \times 10^{-4} \, \text{s/m}^2\text{s}$. The necessary oxygen flux amounts to $(1.36 \pm 0.2) \times 10^{-3} \, \text{s/m}^2\text{s}$ (6.9 moles of oxygen are consumed per mole of toluene). This value is in agreement with the oxygen flux derived from
The hypothesis of an additional layer in biofilms, which protects the bacteria from growth inhibiting or toxic toluene concentrations is corroborated by the qualitative measurement of characteristic exopolymeric substances (EPS) in the biofilm (see Section 4.4.8). These analyses show an accumulation of EPS in the biofilm close to the membrane. The protective effect of natural or artificial gel layers on immobilized cells has also been described by, amongst others, e.g. Costerton (1999) or Keweloh et al. (1989). However, Villaverde et al. (1997b) analyzed an aerobic toluene degrading biofilm, where an inactive layer of biofilm protected the active zone from harmful effects of the toxic substrate. In that case the inactive zone is composed of dead bacteria in the biofilm. This finding of dead bacteria in the protective layer cannot be confirmed in the current study, however. Live/dead analysis of the available biofilm shows a gradual decrease in the ratio of living to dead bacteria, and no increase of the dead fraction, approaching the membrane (see Section 4.4.7).

Another indication for the necessity of a protective layer in the presented system, is the calculated, theoretically possible toluene concentration in the biofilm. The theoretical equilibrium concentration in the first layer of the biofilm, on the membrane, is calculated, using the determined partition coefficient ($H_{maT} = 3.6 \cdot 10^{-3}$) and the toluene concentration in the silicone oil ($c_{aT} = 7598 \text{s/m}^3$, steady-state of operating point number one), amounting to $27.4 \text{s/m}^3$. Although the exact toxicity level of toluene for bacteria cannot be stated, there is evidence that beyond a certain toluene concentration bacterial growth is inhibited. In the selective plate experiments the maximum theoretically possible concentration may reach initially $17 \text{s/m}^3$ (see Subsection 3.9.1.6), where bacteria are able to grow. However, Villaverde et al. (1997a) reported for a $P. putida$ strain, that the fraction of cells, culturable on toluene, decreased drastically after increasing the toluene concentration in the liquid. The maximum concentration tested in that study was only $6.6 \text{s/m}^3$, much lower than the possible concentration on the membrane. It is not possible to conclude whether the toluene concentration reaches already toxic levels, but is has to be assumed that the possible toluene concentration on the membrane are outside the optimal concentration range for bacterial growth.
4.4.2 Biofilm surface-profile / thickness

4.4.3 Surface profile

The thickness of the biofilm is measured by means of a laser distance sensor in situ. The distance between the measuring device and the biofilm surface is acquired through the glass plane of the bio-module and through the nutrient medium. An example of a contour plot, acquired 46 days after start-up, in the course of the steady-state operating condition number one, is shown in Figure 4.22.

The measuring principle works well. The edges and bridges of the static mixers are mirrored in the biofilm surface. At the positions where the membrane is supported by the static mixers, the flux of substrates to the biofilm is reduced. This effect is especially pronounced between two lines of static mixers (see Section 2.2.4), where the supporting edge has a width of 0.5 mm. This smaller feed rate leads to a visibly reduced

![Figure 4.22](image)

Figure 4.22: The contour plot, acquired 46 days after inoculation, shows the biofilm surface structure, with color encoding to indicate biofilm thickness. The dimensions of the analyzed biofilm area are 23x180 mm².
biofilm formation (compare with Figure 4.4). The contour plot detects these areas easily, which are mirrored in the biofilm. Some measured values for the biofilm thickness are less than zero, due to sagging of the membrane. The static mixers used as support have gaps (between the edges) of approximately 5x10mm. The small transmembrane pressure difference used to press the membrane against the static mixers (see Chapter 2.2.4) suffices to let the membranes sag. The calibration of the sensor is made by placing measures of defined thickness on top of the support structure and membrane. There are a few, small areas – in the range of 1 cm² – where temporary biomass accumulations occurs (see Figure 4.22), reaching thicknesses of up to 2 mm.

4.4.4 Thickness

To analyze the biofilm thickness over time, the distinct data points of the biofilm surface scan are averaged to a single value. These single biofilm thickness values are plotted against the time after reactor inoculation (see Figure 4.23). The above mentioned effect of membrane sagging is also well perceptible for the averaged data points. For thin biofilms the average thickness is identified negative. Average thickness varies considerably with time, the steep decrease after 60 days can be explained by a change in the steady-state operating conditions. Up to that point the reactor operates in condition number one with a high toluene surface load of 3.29 $\text{g/m}^2\text{h}$. After the switch in the operation conditions the inlet concentration does not reach as high a value anymore (see Table 3.1). The error bars on the graph represent the standard deviations, calculated for the whole area of scanned biofilm, including the zones of thinner biofilm due to the support structure. The errors are therefore large.

It can be concluded that the measuring principle is well suited for in situ measurements of the biofilm surface structure. However, the support of the membrane should be improved to account for reliable, absolute biofilm thicknesses.

The concept of applying shear stress to the top layer of the biofilm by overflowing nutrient medium, and thereby removing excessive biomass, is
Figure 4.23: The averaged overall biofilm thickness shows considerable fluctuations with time.

a suitable method to hinder biofilm accumulation. The reactor, featuring a channel height of 5 mm, did not clog during the 162 days of operation. This principle of removing biomass by shear stresses was successfully applied by Nicolella et al. (2000a) and Vinage (2002).

The special feature of the present concept, however, is that not the most active top layer of the biofilm is removed, as is the case in the two aforementioned studies, but inactive biomass, which does not contribute to the degradation of toluene.

4.4.5 Estimation of the wall shear-stress

The analysis of the biofilm thickness shows that the bioreactor is at no point during operation in danger of clogging. The culture medium is constantly passing along the biofilm, introducing shear-stress on its top surface and thereby removing excess biomass. This raises the question as to what value the wall shear-stress amounts to in the flat channel. Therefore an estimation based on the Navier-Stokes Equation for an infinite flat channel is made. The coordinate system is placed as follows
4.4. BIOLOGICAL INVESTIGATIONS

(see figure 4.24):

- $x$: flow direction
- $y$: height
- $z$: perpendicular to flow direction

The velocity is denoted: $u = (u, v, w)$.

**Momentum balance (Navier-Stokes Equation)**

$$
\frac{\partial \rho v_i}{\partial t} + v_j \frac{\partial \rho v_i}{\partial x_j} = F_i - \frac{\partial p}{\partial x_i} + \frac{\partial}{\partial x_j} \left( \eta \left( \frac{\partial v_i}{\partial x_j} + \frac{\partial v_j}{\partial x_i} \right) \right)
$$

(4.5)

To be able to solve the equation, the following assumptions are made.

- stationary (fully developed flow)
- the fluid flows between two infinitely extended plates
- the velocity is not dependent on $x$ and $z$
- the pressure is only dependent on $x$, the pressure drop is constant
- gravitational forces are neglected

With these assumptions the Navier-Stokes Equation is reduced to the equation

$$
\frac{\partial p}{\partial x} = \eta \frac{\partial^2 u}{\partial y^2} .
$$

(4.6)

The pressure drop is constant, therefore the following equation is valid

$$
\frac{1}{\eta} \frac{\partial p}{\partial x} = \frac{\partial^2 u}{\partial y^2} = C_1^* ,
$$

(4.7)

which can be integrated with respect to $y$. The velocity profile is then:

$$
u(y) = \frac{1}{2} C_1^* y^2 + C_2 y + C_3 .
$$

(4.8)

The boundary conditions are ($H$: channel height):
• \( u(y = 0) = 0 \)
• \( u(y = H) = 0 \)

It follows that \( C_3 = 0 \) and for the moment \( C_2 = -1/2C_1^*H \). The constant \( C_1^* \) is based on the volume flow, which is found with the aid of the following equation (\( B \): channel width):

\[
\dot{V} = B \int_0^H u \, dy
\]  
(4.9)

For \( C_1^* \) it follows

\[
C_1^* = -\frac{12\dot{V}}{H^3B}
\]  
(4.10)

and for \( C_2 \)

\[
C_2 = \frac{6\dot{V}}{H^2B}
\]  
(4.11)

The equation for the velocity reads therefore

\[ u(y) = C_1 y^2 + C_2 y \]  
(4.12)

whereas \( C_1 = 1/2C_1^* \):

\[
C_1 = -\frac{6\dot{V}}{H^3B}
\]  
(4.13)

The wall shear-stress \( \sigma_w \) is the first derivative of the velocity profile with respect to \( y \):

\[
\sigma_w = \left| \eta \frac{\partial u}{\partial y} \right|
\]  
(4.14)

For the prevailing superficial fluid velocity of \( 6.23 \cdot 10^{-2} \text{m/s} \) and the dynamic viscosity of water at \( 20 \degree \text{C} \), \( \eta = 1 \text{mPa s} \), the wall shear-stress \( \sigma_w(y = 0) \) along the biofilm amounts to 0.074 Pa.

The decrease in channel height due to the biofilm as well as the biofilm surface roughness are not taken into account. Yet, this rough calculation shows that a very small wall shear stress suffices to remove excess biomass, limiting the thickness of the biofilm and thereby preventing the reactor from clogging.
4.4.6 Identification of bacteria

4.4.6.1 Plate counts

Four distinct biofilm samples are taken for bacteria identification procedures. They are sampled 29, 49, 106 and 147 days after reactor start-up. The average number of CFUs per biofilm area is \((1.8 \pm 0.6) \cdot 10^8 / \text{cm}^2\). The large uncertainties are mainly due to the difficult sampling procedure. It is almost impossible to take a biofilm sample without sampling device slipping relative to the membrane, and it is hardly possible to collect the total isolated liquid volume containing the biofilm. Therefore, this number has to be considered as a rough measurement indicating the order of magnitude.

4.4.6.2 Plate selection

Remarkably, an average of only 40 to 50% of biofilm bacteria are still able to grow on toluene (see Figure 4.25). After 30 days the fraction of

Figure 4.24: The calculated velocity profile in the flat channel.
Figure 4.25: The fraction of the culturable bacteria present in the biofilm, able to grow on toluene, is shown for the four biofilm samples, analyzed in the course of the run of the bioreactor.

bacteria that are not able to grow on toluene is already down to approximately 30%. Bacterial strains that are unable to degrade toluene appear rapidly after reactor start and very quickly reach a constant fraction of the total culturable population.

Unfortunately studies on the evolution of the microbial population in biofilms are scarce (Acuna et al., 1999; De Castro, 1996). There are no studies known for membrane based, toluene degrading waste gas treatment systems and few studies were done for biofilters removing toluene from waste gas. Roy et al. (2003) investigated the microbial population of biofilters filled with compost, which were not additionally inoculated with toluene degrading bacterial strains. The number of toluene degraders seems to increase rapidly within the first days after reactor start-up reaching peaks of 20% - 30% before settling to 2% - 9%. Although a different approach is chosen for the procedure of establishing a toluene degrading population in a non-monoseptical bioreactor, this small fraction is surprising. A possible explanation for the difference to the present study could be that in the quoted example toluene is not the only carbon source but also the organic matter in the compost. Acuna
et al. (1999) inoculated a biofilter – analogous to the presented study – with a distinct microbial consortium which used toluene as carbon and energy source. 28 days after inoculation no other bacteria were present. 88 days after inoculation different aerobic bacteria were found, but unfortunately the percentage is not known.

The present system, fed only with toluene and inoculated with two toluene degrading bacterial strains, already acquired a short time after start-up, a remarkable percentage of bacteria that did not have the degradation pathway for toluene at their disposal. These bacteria must feed on biomass, produced by the toluene degraders such as EPS, lysed cells, intermediates or metabolites secreted from the toluene degraders.

Toluene is a bacteriocidic substance (De Smet et al., 1978; Sikkema et al., 1994). Long-term exposure of bacteria to toluene may lead to physiological stress or "injury" of the cells (Hurst, 1977), defined as the physiological, genetic, and structural consequences resulting from sublethal injurious conditions and/or chemical agents. This is manifested by the inability of injured cells to reproduce under selective or restrictive conditions that are tolerated by uninjured cells (McFeters, 1990). The concept of cell injury has been applied in the food and drinking water sectors for many years (Hurst, 1977). In the field of VOC-degradation Leddy et al. (1995) reported the formation of Tol-variants on Pseudomonas putida 54G cultures growing on toluene. These mutants could not degrade toluene anymore. Villaverde et al. (1997a) quantified this nonculturability on toluene of the same P. putida strain and found a pronounced unculturability with increased toluene concentration.

Applying the concept of injured cells to the present analysis has one major impact. The samples from the biofilm are homogenized and spread plated on a complex medium to obtain the total viable count. Single colonies from these plates are picked and successively transferred to selective medium plates. This additional step on the complex medium allows cells, which would have been injured from toluene in the bioreactor, to recover and form colonies on the rich plate as well as on the selective plate. The consequence would be an incorrect fraction of toluene degrading bacteria in the biofilm. However, it has to be countered that: (i) it is not certain whether an injured cell is really able to form a colony on a complex medium, e.g. Villaverde et al. (1997a) showed that the total
number of cells in a biofilm – acquired by cell staining – is considerably higher than the number of total viable cells – acquired by nonselective plate counts; (ii) it is unknown whether a cell, which suffer e.g. from genetic consequences can totally recover on a complex medium to degrade toluene again; and (iii) each measuring principle is subject to some bias. It is possible that a biofilm sample directly plated on a toluene selective medium would have generated a different result. Nevertheless, the current found ratio of toluene culturable cells to total viable cells is in perfect agreement with the aforementioned study (Villaverde et al., 1997a) for toluene concentrations below 5 mg/l, which is the case in the culture medium.

4.4.6.3 Composition of the community

A molecular technique, based on PCR is used to identify the toluene degrading bacteria. Two specific, distinguishable and known DNA sequences for P. putida F1 and R. globerulus PWD1 are amplified. This enables differentiation between the two inoculated strains as well as categorization of newly acquired toluene degraders. Figure 4.26 shows the analyzed composition of the toluene degraders for the four biofilm samples. A large fraction of the toluene degraders consists of strains that are different from P. putida F1 and R. globerulus PWD1. The fractions of these "new" bacterial strains vary between 40% and 70% for the different biofilm samples. This portion seems to peak after approximately 50 days. Furthermore, R. globerulus PWD1 represents only a very small fraction of the toluene degraders, and decreases to a minute proportion already 30 days after inoculation, although the reactor is inoculated with only approximately twice the number of P. putida F1 cells (see Chapter 3.8.3). Due to the disappearance of R. globerulus in the analysis of biofilm sample number two and the reappearance in sample number three, it has to be assumed that this strain is present in the reactor during the entire operation time but always only as a small amount. P. putida F1 represents the major fraction of the toluene degrading bacteria. The fact that P. putida F1 makes up the biggest percentage of the bacterial consortium in the biofilm, is in agreement with comparable literature. Pseudomonas species are reported in several studies on biofil-
It is known that *R. globerulus* is predominant in nutrient limited biofilters. In systems fed with external nutrient sources species such as *P. putida* are favored (Delhomenie et al., 2003; Roy et al., 2003). The membrane attached biofilm in the present system depends only on the nutrient medium. This leads to a possible explanation for the regression of the strain *R. globerulus*. Another explanation is the smaller maximum specific growth rate, $\mu_{\text{max}}$, of *R. globerulus* compared to the one of *P. putida* (see Section 4.4.9). The $\mu_{\text{max}}$ of the strain *R. globerulus* is smaller than that of *P. putida* by approximately a factor of two. Together with the limitation of oxygen in the biofilm, this may contribute to the decrease of *R. globerulus*.

The large fraction of newly acquired toluene degrading bacteria is unexpected, due to the fact, that the reactor is inoculated with only two distinct strains. One or several "new" strains have to be introduced to the reactor via the gas or the water fed to the system, or by unsterile equipment brought in contact with the culture medium. Firstly, it

**Figure 4.26:** The composition of the toluene degrading bacterial fraction in the biofilm is shown for the four biofilm samples.
is generally believed that bacteria in the environment, which have the
degradation pathway of toluene at their disposal, are not very frequent
in air, in contrast to soil or compost, where they readily exist (Roy et
al., 2003). Secondly, these "new" strains must show a similarly good
ability to degrade toluene under the prevailing reactor conditions, other¬
wise they could not have competed with the two inoculated strains and
even displace a considerable fraction of the previous bacterial popula¬
tion. The visual examination of the bacteria grown on plates under
selective conditions – minimal medium with toluene as the only carbon
source – shows that there are at least four different toluene degrading
strains present. One "new" strain forms big, pink colonies, that are very
different from the known colonies formed by P. putida and R. globeru¬
lus. A second strain, which seems newly acquired, forms smaller colonies
that are grayish-green with an almost transparent appearance, and very
difficult to resuspend in water for PCR analysis.

This is the first study on the evolution of the bacterial composition in an
aerobic toluene degrading membrane bioreactor. The numerical fraction
of aerobic bacteria that are not able to degrade toluene amounts to
50%. The composition of the toluene degraders is characterized by a
remarkably big proportion of not identified, newly acquired strains, as
well as by the dominance of P. putida over the remaining part, while
R. globerulus almost vanishes.

4.4.7 Distribution of viable bacteria in the biofilm

The analysis of alive and dead bacteria in the biofilm is done by using
two nucleic acid stains, the green-fluorescent stain SYTO9 penetrates all
bacterial membranes and stains the cells green. In contrast, propidium
iodide (PI) penetrates only bacteria with damaged membranes, redu¬
cing SYTO9 fluorescence when both dyes are present. Due to the fact
that SYTO9 labels all cells, a calibration curve is needed, relating the
fraction of live to dead bacteria to the measured green to red fluorescent
intensities.
The two-point calibration curve is shown in Figure 4.29. A calibration
involving more than two points is deliberately omitted. Takenaka et al.
(2001) as well as the manufacturer of the probes showed that a linear
Figure 4.27: The fraction of alive bacteria is shown versus the live/dead fluorescence ratio.

A relationship exists between the ratio of the fluorescence intensities and the fraction of live bacteria. Analyses of dilutions, made of suspensions of living and dead bacterial cells, will always be more inaccurate than the stock suspensions themselves, featuring 100% living and 100% dead bacteria. The calibration curve exhibits two differences compared to the measurement process, the first being the cells used for the calibration stem from an exponentially growing, planktonic culture and secondly the staining process for this calibration sample is basically different than the one for the biofilm. In the biofilm the cells grow immobilized in a polymeric structure, most likely not in an exponential growth phase and, more important, the cells are stained by dyes that have to diffuse into the biofilm matrix to reach them. The diffusion properties of the stains are not known; it is reasonable to believe, that SYTO9 diffuses faster than PI due to the size of the molecule. In the planktonic culture, the stains are directly mixed to the suspension, which means that the cells are equally surrounded by both dyes.

It has to be concluded that a two-point calibration, based on planktonic cells, is sufficient for the application of the presented live/dead staining kit in biofilms. Due to the discussed unknowns in the course of this
analysis, these results cannot be seen as absolute values, but rather as a
trend.

The green and red fluorescent intensities in the stained biofilm are ac-
quired with an inverted confocal laser scanning microscope (CFLSM) through the transparent PDMS membrane. Figure 4.28 shows a picture of an analyzed slice in the biofilm. The scan is acquired at a depth of 50 \( \mu m \) in the biofilm, measured from the membrane. The averaged re-

![Figure 4.28](image)

**Figure 4.28:** The bacteria in the biofilm are fluorescently labeled with the live/dead stains. They emit green and red fluorescent light. The black lines represent the steel grid, which is incorporated into the membrane. The scan is acquired at a distance of 50 \( \mu m \) from the membrane.

results of the live to dead ratios as a function of the depth in the biofilm are shown in Figure 4.29. The data points represent the ratios, averaged over the analyzed sector of the \( xy \)-plane. The measurements can be carried out to a depth of approximately 100 \( \mu m \). Beyond this distance no light is able to pass into and out of the biofilm due to extinction. The ratio of live to dead bacteria decreases linearly with increasing distance from the membrane. Active bacteria are only present to a depth of about
80 µm from the membrane. This result is in agreement with data stated by e.g. Debeer et al. (1994); Villaverde et al. (1997a). Another interesting result is the course of the graph close to the membrane. Immediately next to the membrane the ratio of living cells is only 50%, although carbon source and oxygen supply should be sufficient to sustain all cells. This might indicate a zone with a reduced fraction of living bacteria due to the harmful interaction of toluene with the cytoplasmic membrane of bacteria (Sikkema et al., 1994). However, this is not likely, because the obtained live/dead ratio decreases at a constant rate from an initial value directly on the membrane to zero at a depth of about 80 µm.

This analysis confirms the generally recognized fact, that only a small fraction of the cells in a VOC degrading biofilm are metabolically active (Arcangeli and Arvin, 1992; Debeer et al., 1994). Considering a total biofilm thickness of 1 mm, the active volume fraction of the biofilm represents 10% at maximum.

This graph only gives evidence about the numerical fraction between the live and the dead bacteria in the biofilm. If the distribution of the
number of the actively, toluene degrading bacteria is investigated, only the green fluorescent intensity has to be considered. Figure 4.30 shows the average measured green intensity in the biofilm as a function of the distance from the membrane. The curve of the green intensity versus the position in the biofilm, distant from the membrane, is an indication for the distribution of the living bacteria. This measure is not normalized with a second intensity measurement, which is acquired at the same time and position in the biofilm, as is the case for the live/dead analysis. This means that it is not corrected for the light extinction. The decrease of the measured green intensity with increasing depth in the biofilm is therefore a combination of a decrease in the number of live cells and an increase of light extinction. However, down to an empirical value of approximately 20 μm, light extinction is generally accepted to be of minor influence on the fluorescence intensity, using living biological specimens. The green fluorescence intensity decreases very rapidly over the first 20 μm micrometers in the biofilm, to reach a value of approximately 1/5 of the initial value at the membrane. This analysis is an indication, that the main fraction of living bacteria is concentrated in a

\[ I_{green} = (64.18 \pm 1.93) \exp[-(7.11e4 \pm 1.19e3)z] + (36.49 \pm 1.85) \exp[-(2.48e5 \pm 1.26e4)z] \]

**Figure 4.30:** The green fluorescence intensity of the live bacteria in the biofilm decreases exponentially with the position in the biofilm. The error bars represent the standard deviations of the individual, averaged measurements.
much thinner layer close to the substrate source. This distribution of living bacteria in the biofilm is incorporated in the model (see Chapter 5). To be able to mathematically describe this distribution of the active biomass an exponential curve is fitted to the measured data. The double exponential curve fit does not claim biological and physical correctness, but it exactly describes the course of the measured data during the first few micrometers of the biofilm.

### 4.4.8 Distribution of EPS in the biofilm – CFLSM

The exopolymeric substances (EPS) in the biofilm, which are mainly composed of polysaccharides (Jahn et al., 1999), are stained with a fluorescent lectin, Concanavalin A (ConA). This lectin binds specifically to D-glucose, D-mannose and sterically related sugars. These sugars are present in the EPS produced by *P. puinida* F1 and *R. globerulus* PWD1 (Iwabuchi et al., 2002; Kachlany et al., 2001; Read and Costerton, 1987), the two inoculating strains. The resulting spatial distribution of the ConA-reactive polysaccharides in the biofilm, analyzed by CFLSM, is shown in Figure 4.31. The normalized, absolute fluorescent intensity in the biofilm is plotted versus the distance in the biofilm from the membrane. The error bars in Figure 4.31 represent the standard deviation of the distinct measurements, therefore the errors decrease with lower fluorescent intensity. However, this progression is deceptive. The total uncertainties have to increase with increasing distance from the membrane, as the unknown influence of the light extinction will thereafter dominate the progression of the uncertainties. The curve describing the concentration of EPS present in the biofilm is therefore only reliable for a qualitative prediction down to a depth of approximately 20 μm.

The curve shows the distribution of the EPS in the biofilm. The curve not only decreases exponentially with increasing distance from the membrane, but it stays constant over the first few micrometers in the biofilm. This is an indication for an accumulation of EPS close to the membrane, explicable on the one hand by the adhesion of the bacteria to the hydrophobic membrane surface (Costerton, 1999; Shreve et al., 1991). On the other hand, toluene is a bacteriocidic substance that harms the cell membranes of bacteria (De Smet et al., 1978; Sikkema et al., 1994). The
cells may protect themselves against toxic toluene concentrations by excreting an EPS layer (Costerton, 1999) onto the membrane, serving as a diffusion barrier. Due to the diffusion process the toluene concentration at the cells is necessarily lowered; from a potentially toxic concentration directly on the membrane, where the toluene concentration may reach the equilibrium concentration with the absorbent (across the membrane), to an ambient one, which is readily metabolized by the bacteria.

### 4.4.9 Maximum growth rate

The maximum growth rate of the toluene degrading bacteria, $\mu_{\text{max}}$, is determined indirectly by measuring the oxygen consumption rate of the bacterial culture. The oxygen transfer rate (OTR) is proportional to the rate of biomass produced per volume of culture medium $dx/dt$.

A typical plot for an experiment carried out with freshly harvested and resuspended biofilm cells is shown in Figure 4.32. The progression of the OTR curve shows a long lag-phase, in the range of 7h, prior to the
exponential growth phase. The bacteria grow exponentially for approximately 5 h on average, before they are limited most likely, by substrate availability. However, it is not clear whether it is oxygen or toluene. Both substrates are fed gaseous and are absorbed by the aqueous phase; neither oxygen nor toluene concentrations can be measured during the run of the experiment. The natural logarithm of the OTR results in an exact straight line for the exponential growth phase. The small standard deviation of the curve fit is a sign of a reliable $\mu_{\text{max}}$ measurement.

The maximum growth rates are evaluated for two biofilm cultures and for the individual inoculating strains *P. putida* F1 and *R. globerulus* PWD1. The values as well as the standard deviations of the distinct measurements of $\mu_{\text{max}}$ are presented in Table 4.3. One of the biofilm samples is freshly harvested from the membrane of the bioreactor and resuspended on day 147 days after inoculation, while the second is pre-cultured for 29 h in a shake flask. Both biofilm samples show a $\mu_{\text{max}}$ value in the same range, which, in spite of the small random uncertainties, are considered to be equal (12% deviation), yet, they show very different lag-phases. Two OTR plots for these different inocula are illustrated in Figure 4.33. The lag phase for the sample, inoculated with resuspended biofilm bac-
Table 4.3: The maximum specific growth rates for two biofilm samples and for the two inoculating strains are shown with the corresponding standard deviations.

<table>
<thead>
<tr>
<th>biofilm consortium</th>
<th>biofilm consortium</th>
<th>P. putida F1</th>
<th>R. globerulus PWD1</th>
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<tbody>
<tr>
<td>resuspended</td>
<td>pre culture 29 h</td>
<td>[1/h]</td>
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<td>0.517 ± 0.016</td>
<td>0.583 ± 0.012</td>
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<td></td>
<td></td>
<td>0.609 ± 0.003</td>
<td>0.383 ± 0.002</td>
</tr>
</tbody>
</table>

Figure 4.33: The lag-phase for the freshly harvested biofilm sample is considerably higher than for the pre-cultured biofilm bacteria.

cell, is five times longer than for the one that has been inoculated with planktonic, pre-cultured biofilm cells. This shortening of the lag phase for the planktonic culture is not surprising, since the cells are already adapted to the culture conditioning.

The maximum specific growth rates for P. putida F1 and R. globeru-
lus PWD1 are also investigated (see Table 4.3). \textit{P. putida} F1 shows a higher \( \mu_{\text{max}} \), while \textit{R. globerulus} PWD1 features a considerably smaller value than the biofilm consortium. The measured \( \mu_{\text{max}} \) value for \textit{P. putida} F1 growing on toluene is slightly lower at approximately 30\% compared with literature data presented for the same strain and substrate (Alagappan and Cowan, 2004; Reardon et al., 2000) (the data of the former, measured at 25\(^\circ\)C and 30\(^\circ\)C, are corrected for differences in culture temperature by linear interpolation). The \textit{R. globerulus} strain not only has a smaller \( \mu_{\text{max}} \), but also a considerably longer lag phase than \textit{P. putida} (see Figure 4.34). The lag phase for \textit{R. globerulus} is approximately three times longer, although both experiments are started with planktonic pre-cultures. This longer lag phase may be an additional explanation for the numerical decline of the strain \textit{R. globerulus} in the biofilm (see Chapter 4.4.6.3).

The maximum specific growth rate, \( \mu_{\text{max}} \), of the biofilm consortium lies between the rates obtained for the two inoculated strains \textit{P. putida} and \textit{R. globerulus} for the available toluene concentration during the Sapromat experiments.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.34.png}
\caption{The OTR for \textit{P. putida} and for \textit{R. globerulus} are compared. \textit{R. globerulus} shows a longer lag phase and a smaller \( \mu_{\text{max}} \).
}
\end{figure}
4.4.10 Biomass production

The produced biomass in a biofilm reactor is composed of bacterial mass and extracellular polysaccharides (EPS). In the following sections the two substance groups are independently analyzed.

4.4.10.1 Production rate of biomass

The rate of produced bacterial biomass is calculated on the basis of the bacterial concentration in the culture liquid and the flow rate of the bleed stream. The concentration of bacterial biomass is determined by gravimetrical analysis. Assuming that there is no net growth or decay of the biofilm, the production rate of bacterial biomass is equal to the rate of discharge of bacteria. Figure 4.35 shows the production rates for the nine steady-state working points. The uncertainties include the standard deviation of the determined mass as well as the systematic uncertainty of the bleed stream. The production rate of bacterial biomass seems to increase with increasing surface load. The influence of the gas flow rate

![Figure 4.35](image-url)
for a distinct surface load on the biomass production rate does not show an unequivocal trend. Therefore, the different values for the production rate of biomass are statistically indistinguishable.

The production rate of EPS is estimated based on total organic carbon (TOC) measurements of the filtrate from the gravimetrical bacterial mass analysis. The organic carbon in the filtrate is determined to be in the range of 50 mg/l. This value is unfortunately within the uncertainty of the measurement device, therefore, the following investigations are done with an estimated error of ±30 mg/l.

4.4.10.2 Elemental composition of the biomass

The elemental composition of the bacteria is analyzed for each steady-state working condition. The probes are investigated for their fraction of carbon, hydrogen and nitrogen. These fractions do not equal one. The remaining fraction is assumed to be only oxygen, since oxygen and ash cannot be distinguished here.

There is no discernible trend in the composition, therefore, the nine results are averaged and the standard deviations are calculated. The obtained composition, taking into account 8% (w/w) "ash" (Doran, 1995), is as follows: $C_{1.93 \pm 0.08}H_{1.16 \pm 0.33}O_{0.13 \pm 0.05}N_0$, with oxygen constituting a large fraction. Comparing the measured elemental composition of the bacteria to an average composition of bacteria grown as planktonic cultures, CH$_{1.8}$O$_{0.5}$N$_{0.2}$ (Doran, 1995), it is found, that the fraction of oxygen increased while the fraction of nitrogen decreased, both by a factor of two. This increase of the oxygen fraction is partly explicable, since it is assumed that not the whole fraction of EPS passed the filter. The polysaccharides, constituting the EPS, are analyzed together with the bacteria, and increase therefore the fractions of C and O compared to N. EPS is assumed to be only composed of sugars with a molar C/O ratio of one, such as glucose for example. The fraction of amino sugars is neglected. However, Kachlany et al. (2001) reported a value of 22% (w/w) N-acetylgalactosamine. Experiments with the biofilm from our set-up using fluorescently labeled lectins, which bind specifically to the glucose, mannose as well as to sterically related sugars, confirm their presence (see Chapter 4.4.8). Therefore, the assumption, considering the EPS as
glucose, featuring a C/O ratio of one, holds true. Kachlany et al. (2001) investigated the composition of EPS, released by a *P. putida* strain and it was found to consist of a molar C/O ratio of 1.07. However, the low C/O ratio (0.75) of the analyzed biomass retained by the filter, is not feasible. The generally accepted stoichiometric formula of bacteria has a C/O ratio of two, and the assumed EPS a ratio of one. The ratio of a combination of these two substances cannot lead to a ratio smaller than one. A possible, simple explanation would be that oxygen alone does not make up for the remaining fraction besides C, H, and N, minerals must be present in the EPS as well.

4.4.11 Yield

The principle of yield coefficients relates the flux of substrate in metabolic pathways to the formation of biomass or different products. Several yield coefficients are commonly used, such as the yield of biomass from substrates $Y_{XT}$, $Y_{XO_2}$ or the yield of product from substrate e.g. $Y_{CO_2S}$.

The yields in the present study are all derived based on the assumption that the bioreactor operates under steady-state conditions. There is no net accumulation or depletion, e.g. the discharged rate of biomass is equal to the biomass production rate.

The true and the apparent yield must be distinguished. The true yield is calculated from the reaction stoichiometry, it is not necessarily equal to the apparent yield, which is observed in the experiment. In biological reactions – which are considered here – cells use substrate for maintenance. In the apparent yield this maintenance is included. Therefore, in the chapters to follow, only apparent yields are considered, and partly included in the model.

4.4.11.1 $Y_{XT}$

The yield of biomass from the substrate toluene, denoted $Y_{XT}$, is defined as the mass of biomass produced per mass of toluene used. In the
present case, \( Y_{XT} \) is calculated dividing the biomass production rate by the toluene consumption rate. The biomass production rate is the sum of the rate of bacterial mass and of EPS contained in the culture liquid, which is carried out of the reactor via the bleed stream. The EPS concentration is assumed to be constant at a value of 50 mg/l (see Section 4.4.10.1). According to the stoichiometric growth and decay reaction (see Equation 4.15) the EPS – which are assumed to feature the C/O ratio of glucose (see Chapter 4.4.10.2) – production rate could be calculated for each steady-state working point. The resulting values would be between 10 g/m³ and 190 g/m³. However, 190 g/m³ would be detectable by means of the TOC analyses of the filtrate, which is not the case. When comparing the uncertainty of the number of CO₂ molecules produced with the average number of EPS (glucose) molecules, it becomes evident that the uncertainty of the CO₂ and the average of EPS are in the same range. Therefore, it is not reasonable to use the stoichiometric growth and decay equation for the determination of the EPS production rate. The constant production rate of bacteria (see Figure 4.35) is evidence for the application of a constant EPS production rate, independent of the gas flow rate and the surface load. The yield calculations are done with a constant concentration of 50 g/m³. The toluene consumption is calculated from a simple mass balance, between inlet and outlet concentration of toluene in the gas stream. The distinct \( Y_{XT} \) values for the nine steady-state working conditions are statistically not distinguishable. Therefore, the averaged \( Y_{XT} \) is calculated to a value, stated in grams dry biomass per grams toluene consumed, of \((0.303 \pm 0.145) g/g\). The bacterial mass accounts only for approximately 20% of the yield. The \( Y_{XT} \) value is in agreement with the value reported by Ergas et al. (1999) of 0.24 g/g for a membrane based toluene degrading bioreactor.

4.4.11.2 \( Y_{XO_2} \)

The yield of biomass from oxygen, \( Y_{XO_2} \), is calculated according to the stoichiometric growth and decay reaction. It is derived based on the yields for bacteria and EPS calculated for the nine steady-state operating conditions. An average stoichiometric Equation (4.15) is assumed for all steady-state working points, since \( Y_{XT} \) as well as \( Y_{CO_2T} \) are virtually
constant:

\[ C_7H_8 + (0.02 \pm 0.01) \text{NH}_4\text{Cl} + (5.24 \pm 0.53) \text{O}_2 \rightarrow (0.14 \pm 0.04) \text{CH}_{1.9}\text{O}_{1.3}\text{N}_{0.1} + (0.77 \pm 0.30) \text{C}_6\text{H}_{12}\text{O}_6 + (3.21 \pm 1.49) \text{CO}_2 + (0.02 \pm 0.01) \text{HCl} + (3.12 \pm 0.13) \text{H}_2\text{O} \]

Toluene is aerobically degraded with ammonium chloride, the nitrogen source in the mineral medium, to biomass, carbon dioxide, hydrochloric acid and water. The uncertainties of the coefficients are the standard deviations, calculated from the coefficients of the distinct 9 equations for each working point. The balances for C and O\(_2\) do not hold exactly for only the averaged values. However, applying a z-test, using the standard deviation (analogous to the carbon balance in Section 4.2.3) the balances for C and O\(_2\) hold, based on a 5\% significance level.

The Equation 4.15 calculates the yield coefficient \(Y_{\text{XO}_2}\) expressed as biomass produced per mass of oxygen consumed. It is \((0.169 \pm 0.083) \text{g/g}\). The uncertainty is derived based on the error margin of the EPS concentration in the nutrient medium of \(\pm 30 \text{g/m}^3\) (see Section 4.4.10.1). This yield coefficient is in agreement with the value of 0.132, stated by Lu et al. (2004).

4.4.11.3 \(Y_{\text{CO}_2\text{T}}\)

The yield of carbon dioxide from toluene, \(Y_{\text{CO}_2\text{T}}\) is calculated based on the measured gaseous and aqueous CO\(_2\) concentrations as well as on the calculated HCO\(_3^-\) content in the culture medium. The coefficient \(Y_{\text{CO}_2\text{T}}\) is shown for the nine different steady-state operating conditions in Figure 4.36. The nine distinct values obtained in the course of the steady-state operating conditions are statistically indistinguishable. The average value is therefore \((3.21 \pm 1.49) \text{mol/mol}\). Approximately three moles of carbon dioxide are produced per mole of toluene consumed. In spite of the large total uncertainties, the measurements show a trend to smaller yields for higher surface loads. This course is explicable as all yields are relative measures. The fraction of CO\(_2\) produced by maintenance gains importance for decreasing loads, compared to the fraction of CO\(_2\) produced...
Figure 4.36: The yield of carbon dioxide from toluene is shown on a molar basis for the nine steady state working conditions as a function of the surface load and the gas flow rate.

during cell growth, which decreases with reduced substrate availability (see Figure 4.35).
Chapter 5

Modeling of the novel waste gas treatment system

A mathematical model of the novel waste-gas treatment set-up is developed. Modeling is done for mainly three reasons.

- The complex concept of the pilot plant requires a model for the dimensioning of the reactor. This first simpler model is not shown.

- A more complex model – the one discussed in this chapter – includes parameters stemming from independent experiments and the present reactor. This model is used to point out the important parameters for the performance of the installation.

- To write a model is to use it for scale-up considerations.
5.1 Introduction to modeling of membrane attached biofilms

The behavior of biofilms is determined by biological, chemical, and physical processes internal to the biofilm as well as by the interaction of the biofilm with its environment. Several mechanistic models have been developed, describing the conversion of substrate by biofilms using a continuum approach (Skowlund, 1990; Wanner, 1995). Often, simplifying assumptions are made to be able to solve the differential equations analytically (e.g. first-order or zero-order growth kinetics with respect to the biomass concentration) (Ergas et al., 1999). More complex studies, using numerical solutions, incorporated e.g. growth, substrate inhibition, decay and death rates (Mirpuri et al., 1997; Villaverde et al., 1997b). The derived equations rely on mass balance principles and on the a priori assumption that all components considered in the model (chemical substance, microorganisms, etc.) may be treated as continuum rather than as individual particles (Characklis and Marshall, 1990). Unfortunately, this continuum assumption is often expanded not only to distinct particulate matter but to the whole biofilm, e.g. the homogeneous biomass distribution along the biofilm thickness. Very few studies included microbial spatial distributions (Characklis and Marshall, 1990). Only a few models have so far been developed that describe the mass transfer and degradation processes occurring in membrane attached biofilms, where counter-diffusion of substrates in the biofilm is the norm (Ergas et al., 1999; Nicolella et al., 2000b).

5.2 Model equations

The model considers four different phases: gas, absorbent, biofilm and nutrient medium. The phases are in contact with each other and at their boundaries the prevailing two phases are in equilibrium. The membranes separating the four phases from each other are not explicitly included in the model. They appear indirectly in the corresponding overall mass transfer coefficients. This course of action is chosen, because neither the
concentration in the fluid layer next to the membrane, nor the concentrations in the membrane are known. However, these concentrations are necessary to explicitly model the mass transfer across the membranes. In contrast, the overall mass transfer coefficients can be determined experimentally. For each phase, time dependent differential equations for the concentrations of toluene and oxygen are derived. For the gas as well as for the biofilm phase the differential equations are written locally one-dimensional. The model equations are solved by use of a commercially available ordinary differential equation (ODE) solver (Berkeley Madonna, Version 8.0.1 Robert I. Macey & George F. Oster). The use of an ODE solver requires manually discretizing the spatial variable in the gas phase along the $x$-direction and in the biofilm phase along the $z$-axis.

5.2.1 Gas phase

The gas phase, consisting of air enriched with toluene, is modeled one-dimensionally, with orthogonal mass exchange. The gas streams in the $x$-direction, the compounds are transported by convection and diffusion. Toluene and oxygen are exchanged between the gas and the silicone oil during the flow of the gas along the membrane. The mass transferred from the gas into the silicone oil is modeled assuming the two phases to be in equilibrium with each other. The membrane is only seen as an additional resistance, without influencing the thermodynamical equilibrium between the gas and the absorbent. The applied mass transfer coefficient in the model is measured from one bulk fluid to the other.

5.2.1.1 Differential equation

The derivation of the differential equation, starting from a simple mass-balance for an infinitesimal element, is shown here for the gas channel
Figure 5.1: The schematic of the model system shows the four phases of the reactor, the gas, the absorbent, the biofilm and the nutrient medium. The phases are in equilibrium with each other at the individual boundaries. For each phase differential equations for the concentration of toluene and oxygen are derived. The equations are written time dependent, and for the gas phase as well as for the biofilm phase locally one-dimensional. The dotted lines mark sections of infinitesimal elements used for the manual discretization of the spatial variable.
5.2. MODEL EQUATIONS

and is substitutional for all other phases.

\[
\frac{\partial M^{S_i}}{\partial t} = \left( j^{\text{conv},S_i} \bigg|_x - j^{\text{conv},S_i} \bigg|_{x+dx} \right) (b \cdot h)
\]

\[
+ \left( j^{\text{diff},S_i} \bigg|_x - j^{\text{diff},S_i} \bigg|_{x+dx} \right) (b \cdot h)
\]

\[
- j^{\text{abs},S_i} \bigg|_x (b \cdot dx)
\]

\hspace{1cm} (5.1)

In Equation 5.1 \( b \) and \( h \) denote the width and the height of the gas channel.

The mass transport is governed by three fluxes: the fluxes in the gas-phase into and out of the infinitesimal element, one driven by convection, the other induced by diffusion as well as by the absorbed mass flux. The diffusion is modeled according to Fick’s first law. The individual fluxes are described in the following equations (5.2).

\[
j^{\text{conv},S_i} = \bar{v}^g \cdot c^{gS_i}
\]

\hspace{1cm} (5.2a)

\[
j^{\text{diff},S_i} = -D^{S_i} \frac{\partial c^{gS_i}}{\partial x}
\]

\hspace{1cm} (5.2b)

\[
j^{\text{abs},S_i} = \dot{m}^{gS_i} = k^{aS_i} \left( \frac{1}{H^{gS_i}} c^{gS_i} - c^{aS_i} \right)
\]

\hspace{1cm} (5.2c)

where \( \bar{v}^g \) denotes superficial gas velocity, \( D^{S_i} \) the diffusion coefficient of the substrate in question, \( c^{gS_i} \) the substrate concentration in the gas. The overall mass transfer coefficient \( k^{aS_i} \) is determined from bulk fluid to bulk fluid and based on the absorbent-side. The partition coefficient of the compound between the gas-phase and the absorbent-phase is denominated \( H^{gS_i} \) while \( c^{a} \) stands for the substrate concentration in the absorbent. These three expressions substituted into Equation 5.1 lead
to the following equation:

\[
\frac{\partial M_{S_i}}{\partial t} = \left( \frac{v^g c^{S_i}}{x} - \frac{v^g c^{S_i}}{x+dx} \right) (bh) + \left( -D \frac{\partial c^{S_i}}{\partial x} \bigg|_x + D \frac{\partial c^{S_i}}{\partial x} \bigg|_{x+dx} \right) (bh) \\
- k_{ov}^{aS_i} \left( \frac{1}{H_{gas_i}} c^{S_i} - c^{aS_i} \right) \bigg|_x (bdx)
\]  

(5.3)

Assuming that Equation 5.3 is continuous in the closed interval [0,1] and that within this interval the function holds derivatives up to the order \(n-1\), where \(n\) derivatives exist, then the Taylor series expansion is applicable.

\[
f(c^{S_i})|(x+dx) = f(c^{S_i})|_x + \frac{f'(c^{S_i})}{1!} \bigg|_x ((x + dx) - x) + \cdots
\]

By applying this series expansion to the above stated equation, the following expression is obtained.

\[
\frac{\partial M_{S_i}}{\partial t} = bh v^{gS_i} \left( c^{S_i} \bigg|_x - \left( c^{S_i} \bigg|_x + \frac{\partial c^{S_i}}{\partial x} \bigg|_x \right) dx \right) + bh \left( -D \frac{\partial c^{S_i}}{\partial x} \bigg|_x + D \frac{\partial c^{S_i}}{\partial x} \bigg|_x + D \frac{\partial^2 c^{S_i}}{\partial x^2} \bigg|_x dx \right) \\
- k_{ov}^{aS_i} \left( \frac{1}{H_{gas_i}} c^{S_i} - c^{aS_i} \right) \bigg|_x (bdx)
\]  

(5.4)

Dividing Equation (5.4) by the volume of the infinitesimal element finally leads to the desired differential equations for the compounds in the gas phase of the model:

\[
\frac{\partial c^{S_i}}{\partial t} = -v^g \frac{\partial c^{gS_i}}{\partial x} + D \frac{\partial^2 c^g}{\partial x^2} - \frac{1}{h} k_{ov}^{aS_i} \left( \frac{1}{H_{gas_i}} c^{gS_i} - c^{aS_i} \right)
\]  

(5.5)
5.2. MODELEQUATIONS

5.2.1.2 Discretization of the differential equation

The gas-phase itself can be solved analytically for steady-state conditions, however, this proves to be difficult for dynamic conditions. It is nearly impossible to find an analytical solution of the differential equations describing all four phases of the reactor under dynamic conditions. A numerical solution calculated with an ODE solver requires a manual discretization of the spatial variable of the partial differential equation. The first derivative is described using a backward, two-point, finite-difference approximation; the discretized form of the second derivative is obtained by the central finite-difference approximation (Oezisik, 1994) of Equation 5.5.

This leads to the final equation which is used for the modeling of the gas phase:

\[
\frac{\partial \varepsilon \bar{g} \delta_{j}^S_{i}}{\partial t} = -\bar{g} \varepsilon_{i}^g \delta_{j}^S_{i} - c_{i-1}^{gS_{i}} \frac{\Delta x}{\Delta x} + D \frac{c_{i-1}^{gS_{i}} - 2c_{i}^{gS_{i}} + c_{i+1}^{gS_{i}}}{(\Delta x)^2} \nonumber \\
- \frac{1}{h} k^{aS_{i}} \left( \frac{1}{H^{aS_{i}}} c_{i}^{gS_{i}} - c_{aS_{i}}^{a} \right) \nonumber \\
+ O_1(\Delta x) + O_2((\Delta x)^2)
\]

where \( O_1(\Delta x) \) respectively \( O_2((\Delta x)^2) \) characterize the truncation errors associated with the finite difference approximation of the first, respectively the second derivative. \( \Delta x \) is the displacement of the grid points along the length of the absorption module.

\[
O_1(\Delta x) \equiv \frac{\Delta x}{2} f''(c^g)|_x + \frac{(\Delta x)^2}{6} f'''(c^g)|_x + \cdots
\]

\[
O_2((\Delta x)^2) \equiv \frac{(\Delta x)^2}{12} f''''(c^g)|_x + \cdots
\]

5.2.1.3 Assumptions

The model of the gas-phase is based on the following assumptions:
• The gas is ideally mixed along the height and along the width of the channel.

• The velocity profile of the gas is of the form plug-flow.

• The equilibrium between the gas and the absorbent is not altered by the presence of the membrane.

• The model is only solved for the case where the substrates in the gas are transported by convection (it will be shown later that the diffusion is of no influence, and it is therefore omitted in the calculations).

5.2.2 Absorbent phase

The absorbent phase is modeled as an ideally mixed tank. The absorbent is in equilibrium with the gas phase and with the biofilm. The absorbent is loaded with toluene and oxygen removed from the gas phase. The desorption of the two substrates is carried out by biological degradation in the biofilm. The mass transfers to and from the absorbent phase are modeled in equilibrium with the adjacent gas and water phases irrespective of the two separating membranes.

5.2.2.1 Model equations

Since the absorbent phase is modeled as one ideally mixed tank, the differential equation for the compound in question has to hold for the whole balance region. The concentration in the absorbent changes with time by exchanging the mass flux $\dot{M}^{ga}$ with the gas phase and by exchanging the mass flux $\dot{M}^{ab}$ with the biofilm phase:

$$\frac{\partial c_{aS_i}}{\partial t} = \frac{1}{V_a}(\dot{M}^{gaS_i} - \dot{M}^{abS_i}).$$ (5.7)
These fluxes are described by the equations 5.8,

$$
\dot{M}_{gaS_i} = \int_{0}^{l_{abs}} \left( \frac{1}{H_{gaS_i}} c_{gaS_i}(x) - c^{asS_i}_i \right) b \, dx (5.8a)
$$

$$
\dot{M}^{abS_i} = k^{asS_i}_o \left( c^{asS_i}_i - \frac{1}{H_{amasS_i}} c^{bsS_i}_i \right) (b l_{bio}) (5.8b)
$$

$l_{abs}$ and $l_{bio}$ denote the length of the absorbent and the biofilm module, respectively. Both modules have the same width $b$. $H_{am}^{S}$ is the partition coefficient of the compound in question between the absorbent-phase and the aqueous-phase, where "aqueous phase" denotes the biofilm as well as the nutrient-medium. $c^{bsS_i}$ stands for the concentration of the considered compound in the biofilm.

### 5.2.2.2 Discretization of the differential equation

The discretized differential equations describing the concentration changes in the absorbent phase are shown in Equation 5.9:

$$
\frac{\partial c^{asS_i}}{\partial t} = \sum_{i=0}^{N} \left( \frac{1}{H_{gaS_i}} c_{gaS_i} - c^{asS_i}_i \right) b \Delta x - \dot{M}^{abS_i}_i
$$

$$
= \sum_{i=0}^{N} k^{asS_i}_o \left( \frac{1}{H_{gaS_i}} c_{gaS_i} - c^{asS_i}_i \right) b \Delta x - k^{asS_i}_o \left( c^{asS_i}_i - \frac{1}{H_{amasS_i}} c^{bsS_i}_i \right) (b l_{bio}) (5.9)
$$

where $N$ denotes the number of grid points along the length of the absorption module in the gas phase.

### 5.2.2.3 Assumptions

The modeling of the absorbent-phase is based on the following assumptions:
• The absorbent is ideally mixed within the whole tank. It is clear from a scientific point of view that this assumption does not exactly hold for the real set-up. In the available reactor the absorbent flows in a loop from tank one through the absorption module to an intermediate tank two. From this tank one part of the absorbent flows directly back to the first tank and the other part flows through the bio-modules back to tank one. Along the way, the concentrations of the compounds in question have to change, otherwise the set-up would not work. However, the assumption can be justified because of the high flow rate of the absorbent. The concentration of the absorbent does not change considerably during one flow cycle through the bioreactor module. The concentration difference between the beginning and the end of the cycle is hardly detectable. A simple mass balance for the desorption shows that even for a reported high biological degradation rate of $1.5 \times 10^{-4} \text{g/m}^2\text{s}$ (Vinage and von Rohr, 2003) an exemplary concentration of $3'000 \text{g/m}^3$ toluene in the absorbent does not change by more than 0.25% after one pass through this module. (The example is calculated based on a mean residence time of the absorbent in the biofilm module of 400s, on a biofilm area of 0.275m$^2$ and on an absorbent volume in the modules of $2.29 \times 10^{-3} \text{m}^3$.) On the other hand there are always stagnant regions in a reactor and in the static mixers which may lead to unavoidable concentration gradients.

• The absorbent is in equilibrium with both the gas and the biofilm.

• The overall mass transfer coefficients for the transfer of toluene and oxygen from the bulk absorbent to the first layer on the membrane in the aqueous phase is taken on from the experimentally determined coefficient for the mass transfer from the bulk gas to the bulk absorbent.

5.2.3 Biofilm

The biofilm is modeled one dimensional, with normal mass exchange along the z-axis. The mass within the biofilm is only transported by diffusion. The investigated compounds, toluene and oxygen, are aerobically
degraded by bacteria. The mass of actively toluene degrading bacteria is not homogeneously distributed in the whole biofilm; it is distributed according to experimentally derived data relating to the distribution of the active bacteria along the thickness of the biofilm. These data stem from the fluorescent intensity measurements of the green fluorescently stained active bacteria in the biofilm (see Chapter 3.9.2.4). This measured green fluorescent intensity profile along the thickness of the biofilm is denoted as \( I_{\text{green}}(z) \). The total bacterial mass in the bioreactor contributing to the toluene degradation is calculated based on results from the plate count experiments with an assumed geometrical form and an assumed dry mass fraction of the bacteria. The degradation rates of toluene and oxygen are assumed to be directly related to the bacterial growth rate and the biomass concentration by the yield coefficients. The growth rate is modeled following a double Monod kinetic based on the toluene and oxygen concentrations. The model is written to take only the toluene degrading bacteria into account, although it has been shown that only approximately half of the number of all aerobic, culturable cells are able to grow on toluene. The other half is assumed to use secondary metabolites as growth substrates. However, since there is no information about the growth kinetic of these bacteria, it is not possible to include their contribution to the oxygen consumption into the model.

### 5.2.3.1 Model equations

The mass flux \( \dot{M}^{\text{abs}} \) exchanged between the absorbent phase and the biofilm is described by Equation 5.8. The mass transferred from the biofilm to the nutrient medium \( \dot{M}^{\text{bms}} \) is defined by

\[
\dot{M}^{\text{bms}}_i = \beta (c^{\text{bs}_i} - c^{\text{ms}_i}) (b_{\text{bio}}).
\]  \tag{5.10}

The equation describing the concentrations of the investigated substrates in the biofilm reads as follows:

\[
\frac{\partial c^{\text{bs}_i}}{\partial t} = D^{\text{si}} \frac{\partial^2 c^{\text{bs}_i}}{\partial z^2} - r^{\text{si}},
\]  \tag{5.11}
where $r_{Si}^S$ is the substrate removal rate.
This removal rate is related to the growth rate by the following equation:

$$r_{Si}^S = \frac{\mu}{Y_{XSi}} x^b$$

(5.12)

$\mu$ is the specific growth rate of the toluene degrading bacteria in the biofilm, $Y_{XSi}$ is the biomass yield and $x^b$ stands for the concentration of the mass of cells in the biofilm.

The growth rate $\mu$ is dependent on the substrate concentrations. In the present case, it is not obvious which is the limiting substrate, therefore a double Monod kinetic approach is chosen, depending on toluene ($T$) and oxygen ($O_2$).

$$\mu = \mu_{max} \left( \frac{c^{bT}}{K_T^S + c^{bT}} \right) \left( \frac{c^{bO_2}}{K_{O_2}^S + c^{bO_2}} \right)$$

(5.13)

where $c^{bT}$ and $c^{bO_2}$ are the toluene and oxygen concentrations available to the cells and $K_T^S$ and $K_{O_2}^S$ the half-saturation constants for these substrates.

### 5.2.3.2 Discretization of the differential equation

The non-homogeneous distribution of the bacterial mass in the normal direction in the biofilm requires a non-uniform, higher resolved mesh close to the membrane, with $z_j = \cos \theta_j$ for $\theta_j = (j-1)(\pi/2)/(P-1)$, $j = 1, 2, \ldots, P$. Here $P$ is the number of grid points in the $z$-direction.

Due to the non-uniform grid spacing, the discretized form of Equation 5.11 for grid points $j = 2 \ldots P - 1$ is of the following form (where the denotation of the exact substrate is omitted for simplicity):

$$\frac{\partial c_j^{bS_i}}{\partial t} = -D_i^S (c_j^{bS_i} - c_{j-1}^{bS_i}) / 0.5(z_{j-1} + z_j) + D_i^S (c_{j+1}^{bS_i} - c_j^{bS_i}) / 0.5(z_j + z_{j+1}) - r_j^{bS_i}$$

(5.14)

The mass transferred from the absorbent to the biofilm is delivered to the first slice, therefore Equation (5.14) changes for $j = 1$ to the following
form:
\[
\frac{\partial c_{1}^{bS_i}}{\partial t} = k_{ov}^{as_i} (c_{aS_i} - \frac{1}{H_{amS_i}}c_{1}^{bS_i}) b_{bio} + \frac{D_{S_i}^{bS_i}(c_{2}^{bS_i} - c_{1}^{bS_i})}{0.5(z_1 + z_2)} (5.15)
\]

It is noteworthy that the thickness of the first slice is arbitrarily set to a very small value of only \(10^{-9} \mu m\).

For \(j = P\) Equation 5.14 changes to
\[
\frac{\partial c_{P}^{bS_i}}{\partial t} = -\frac{D_{S_i}^{bS_i}(c_{P}^{bS_i} - c_{P-1}^{bS_i})}{0.5(z_{P-1} + z_{P})} - \beta^{mS_i}(c_{P}^{bS_i} - c_{mS_i}) \frac{b_{bio}}{z_{P}} - r_{P}^{bS_i} (5.16)
\]

Since the biomass is heterogeneously distributed along the \(z\)-axis in the biofilm, Equation 5.12 is written in the discretized form as follows:
\[
r_{j}^{bS_i} = \frac{\mu_{j}^{b}}{Y_{XS_i}} x_{j}^{b} (5.17)
\]

The growth rate \(\mu_{j}^{b}\), depending on the position in the biofilm, is defined by Equation 5.18:
\[
\mu_{j}^{b} = \mu_{max} \left( \frac{c_{j}^{bT}}{K_{S}^{T} + c_{j}^{bT}} \right) \left( \frac{c_{j}^{bO_{2}}}{K_{O_{2}}^{b} + c_{j}^{bO_{2}}} \right) (5.18)
\]

### 5.2.3.3 Assumptions

The model of the biofilm is only valid under the following listed assumptions:

- The biofilm is modeled as a dense, pore-free body.
- The biofilm has a fixed thickness.
- The mass transport is only carried out by molecular diffusion.
• The diffusion coefficients for both investigated substrates are constant throughout the whole biofilm.

• The active bacterial mass in the biofilm is heterogeneously distributed in the normal direction. The bacteria concentration profile over the biofilm thickness is adopted from Live/Dead Confocal Laser Scanning Microscopy analysis in the real biofilm.

• The biomass concentration is assumed to be homogeneous over the whole area of the biofilm for each distinct depth.

• The number of active substrate degrading bacteria is constant over time, although a bacterial growth kinetic is introduced.

• The bacterial growth follows Monod kinetic.

• The degradation rates of the investigated substrates are directly related to the growth rate of the bacteria by the yield coefficients.

• The production and occurrence of exopolymeric substances (EPS) in the biofilm is not included in the model.

• The only potentially growth limiting substances (substrates as well as reaction products) are toluene and oxygen.

• The biofilm is in equilibrium with the absorbent and the mineral medium at the two individual interfaces. Equilibrium is assumed neglecting the membranes.

• The overall mass transfer coefficient between gas and absorbent has the same value as the one between absorbent and biofilm (see Chapter 5.2.2.3).

• The partition into four subunits of the biodegradation module of the real system is not taken into account in the model.

5.2.4 Nutrient medium

The nutrient medium phase is modeled as a continuously stirred tank reactor. The bulk volume is ideally mixed. A constant feed stream
delivers fresh, oxygen saturated mineral medium to the tank. A bleed stream discharges bulk liquid at the same flow rate as the feed is supplied. Bacteria suspended in the culture medium degrade the substrates.

5.2.4.1 Model equations

The substrate concentrations in the mineral medium vary with time by exchanging the mass flows $\dot{M}^{\text{bm}S_i}$ with the biofilm, by the convective flows $\dot{M}^{\text{feed}S_i}$ and $\dot{M}^{\text{bleed}S_i}$ as well as by the biological degradation $r^{S_i}$ (Equation 5.19).

$$\frac{\partial c^{mS_i}}{\partial t} = \frac{1}{V^m} \left( \dot{M}^{\text{bm}S_i} + \dot{M}^{\text{feed}S_i} - \dot{M}^{\text{bleed}S_i} \right) - r^{S_i} \quad (5.19)$$

These rates for toluene and for oxygen are described by the following equations:

$$\dot{M}^{\text{bm}S_i} = \beta^{mS_i} \left( c^bS_i - c^{mS_i} \right) b l_{\text{bio}} \quad (5.20a)$$

$$\dot{M}^{\text{feed}S_i} = V^m c_0^{mS_i} \quad (5.20b)$$

$$\dot{M}^{\text{bleed}S_i} = V^m c^{mS_i} \quad (5.20c)$$

$$r^{mS_i} = \frac{\mu^m}{Y_{XS_i}} x^m \quad (5.20d)$$

where $\mu^m$ is the specific growth rate of the bacteria in the mineral medium, analogous to Equation 5.13 for the biofilm; $x^m$ is the concentration of suspended bacteria in the mineral medium; $Y_{XS_i}$ is the growth yield in the liquid culture and $c_0^{mS_i}$ is zero where $S_i$ denotes toluene.

5.2.4.2 Discretization of the differential equation

Only the last element of the biofilm is in equilibrium with the mineral medium, therefore the balance equation is written as follows:

$$\frac{\partial c^{mS_i}}{\partial t} = \frac{1}{V^m} \left( \beta^{mS_i} \left( c_P^{bS_i} - c^{bS_i} \right) \left( b l_{\text{bio}} \right) + V^m \left( c_0^{mS_i} - c^{mS_i} \right) \right) - r^{mS_i} \quad (5.21)$$
5.2.4.3 Assumptions

- The nutrient medium is ideally mixed in the whole reservoir.
- The substrate concentrations in the bleed stream are the same as in the bulk fluid.
- The nutrient medium is sealed off against the environment, except for the feed and the bleed stream. (Although this is the same case for the remaining three phases it is specially noteworthy for the culture medium. No oxygen from the surrounding environment can be absorbed by the nutrient medium).
- The bacterial growth follows Monod kinetics.
- The only substances which possibly limit the bacterial growth are toluene and oxygen. All the other nutrients are available for the bacteria in excess and no reaction products inhibit growth.
- The degradation rate is related to the growth rate by the yield coefficient.
- The maximum degradation rate is the same as in the biofilm.

5.2.5 Model parameters

The parameters used in the above described model are separated into three groups: (i) specifying the dimensions of the modeled real reactor, (ii) stating the used physical coefficients of the phases and (iii) listing the microbiological parameters. The parameters are summarized in Appendix B.

5.2.5.1 Dimension parameters

Unless otherwise stated the parameters are taken from the existing reactor.
5.2. MODEL EQUATIONS

**Gas phase**

\( V_g \quad \text{The volume of the gas phase in the absorbent module 0.911.} \)

\( \dot{V}_g \quad \text{The standard operating gas flow rate is set to } 1 \, \text{lm}^{-1} \text{min}^{-1}. \text{ This parameter is varied in the range of 1 to } 4 \, \text{lm}^{-1} \text{min}^{-1}. \)

\( A_g \quad \text{The absorption area amounts to } 0.19 \, \text{m}^2. \)

\( N \quad \text{The number of grid points used in the normal direction to discretize the gas phase is set to } 50. \)

**Absorbent phase**

\( V_a \quad \text{The total volume of the absorbent filled into the reactor is 71.} \)

\( A_a = A_g \quad \text{The area provided for the absorption on the absorbent side of the membrane is set to } 0.19 \, \text{m}^2 \text{ as it is also used for the area on the gas side of the membrane.} \)

**Biofilm**

\( h_b \quad \text{The thickness of the biofilm is set to a constant value of } 1 \, \text{mm.} \text{ This value is in the range of the measured average biofilm thickness.} \)

\( A^b \quad \text{The area of the biofilm is set to the sum of the membrane areas available for biofilm growth in the four bio-modules. The area is } 0.27 \, \text{m}^2. \)

**Nutrient medium**
$V^m$ The volume of the nutrient medium includes both tanks, the piping, the pump head and the free volumes in the four bioreactor modules, totaling 101.

$\dot{V}^m$ The feed and the bleed streams are set to 0.209 l/h.

5.2.5.2 Physical parameters

$T$ The temperature is not explicitly included in the model. It is used to calculate the partition and diffusion coefficients, which are temperature dependent. The temperature is set analogously to the one measured in the reactor; the gas is set to 25 °C, the average of the inlet and the outlet gas temperatures measured, the absorbent and the culture medium are set to 28 °C.

Partition coefficients

$H^{gaT}$ The toluene air-absorbent partition coefficient is interpolated for a temperature of 25 °C and for a final concentration of 5000 g/m³. It amounts to $1.062 \cdot 10^{-3} \pm 5.31 \cdot 10^{-5}$.

$H^{gaO_2}$ The measured and applied value for the partition coefficient is 5.04 with an estimated error of ± 0.5.

$H^{maT}$ The partition coefficient between the absorbent and the biofilm is not measurable experimentally. The used partition coefficient is measured with water, it is $3.6 \cdot 10^{-3}$ with a calculated error of $7 \cdot 10^{-4}$.

$H^{maO_2}$ The measured coefficient used in the model is 0.12 with an estimated error of ±10%.

Mass transfer coefficients
\( k_{ov}^{aT} \) denotes the overall mass transfer coefficient for toluene through the membrane. It is based on the absorbent side and describes the mass transfer from bulk gas to bulk absorbent, including the mass transfer resistances due to boundary layers on both sides of the membrane as well as the resistance due to diffusion through the membrane. It is experimentally determined to be \( 4.0 \cdot 10^{-3} \text{ m/h} \) with a calculated error of \( 1.2 \cdot 10^{-3} \text{ m/h} \).

The same value is used for the mass transfer through the second membrane, separating the absorbent from the biofilm. It qualifies the mass transfer from the bulk of the absorbent to the first aqueous layer on the membrane. This application of the same coefficient for both systems – gas absorbent as well as absorbent aqueous phase – implies the assumption of the mass transfer resistance on the gas side of the membrane to be negligible. Therefore the same coefficient is used describing the mass transfer between the bulk absorbent and the bulk gas, as well as between the bulk absorbent and the first layer on the membrane on the aqueous side (see Chapter 3.4.3 for information about the measurement procedure).

\( k_{ov}^{aO_2} \) The overall mass transfer coefficient for oxygen from the gas to the absorbent, based on the absorbent side, is experimentally determined \( 6.7 \cdot 10^{-2} \text{ m/h} \) with a possible range of \( 4.9 \cdot 10^{-3} \text{ m/h} \) for a membrane thickness varying between 80 and 150 \( \mu \text{m} \).

This mass transfer coefficient for oxygen is applied to both membranes as is the case for the coefficient describing the mass transfer across the membrane for toluene.

\( \beta_{mT} \) The mass transfer coefficient describing the transfer of toluene from the biofilm to the nutrient medium is estimated using a \( Sh \)-correlation (Stephan, 1960) in the range of \( 1.3 \cdot 10^{-2} \) to \( 1.5 \cdot 10^{-2} \text{ m/h} \), while the former is used as the standard model parameter.

\( \beta_{mO_2} \) The mass transfer for oxygen from the biofilm to the nutrient medium is estimated using the same \( Sh \)-correlation stated above
(Stephan, 1960). The Schmidt-number $Sc = 337$ is taken from the Table stated by Ramsing and Gundersen (2004) for the appropriate salinity of M9 minimal medium and a temperature of $28^\circ$C. The Reynolds ($Re$) number is calculated using the same parameters as done for $\beta^{mT}$. The mass transfer coefficient $\beta^{mO_2}$ is set to $3.0 \cdot 10^{-2} \, m/h$.

5.2.5.3 Diffusion coefficients in biofilms

The diffusion coefficients vary in biofilms with age of the film, location, as well as the properties of the biofilm, such as density or porosity (Wanner, 1995). These variations are partially overcome by introducing averaged, effective diffusion coefficients. However, in case of convection in pores of the biofilm the effective coefficient is higher than the diffusion coefficient in water, and for biofilms featuring high densities, the effective diffusion might be lower. These effective diffusion coefficients therefore also vary therefore from one location to another, and decrease towards the bottom of the biofilm (Beyenal et al., 1998; Bishop et al., 1995). Although there is currently no literature published on studies of diffusion coefficients in biofilms grown on membranes, it is reasonable to assume constant diffusion coefficients in the biofilm.

$D^{bT}$ This parameter is taken from literature where it is described to vary between 30\% to 200\% of the value in water (Arcangeli and Arvin, 1992; Characklis et al., 1982; Libicki et al., 1988; Siegrist and Gujer, 1985; Zhang et al., 1998). In the model a diffusion coefficient of $3.9 \cdot 10^{-6} \, m^2/h$ is chosen. It amounts to 80\% of the diffusion coefficient in water. This value is often applied for fixed film reactors (e.g. Bibeau et al., 1997; Ottengraf and Vandenoevever, 1983; Parker et al., 1997).

$D^{bO_2}$ This parameter is taken on from comparable literature. The diffusion coefficient of oxygen in active biofilms is reported to vary between 4.8 and 70\% (Beyenal et al., 1997; Stewart, 1998, 2003) of the coefficient in water. The model is calculated with a value of $8.64 \cdot 10^{-6} \, m^2/h$. This value corresponds to 40\% of the oxygen diffusion coefficient in water.
featuring a salinity of 11.16 s/i – M9 minimal medium – at a temperature of 28 °C (Ramsing and Gundersen, 2004). This percentage range is reported by Beyenal et al. (1998) for a mono-species biofilm composed of *Pseudomonas* specie.

5.2.5.4 Oxygen solubility

$c_0^{mO_2}$ The oxygen concentration in the fresh nutrient medium, fed to the reactor, is set to 7.36 g/m$^3$. This is the equilibrium oxygen concentration of water in contact with air at a temperature of 28 °C and featuring a salinity of 11.16 s/i (Ramsing and Gundersen, 2004).

5.2.5.5 Half-saturation constants

The half-saturation constants could not be evaluated experimentally within the scope of this work and are taken from existing literature.

$K_S^T$ The half-saturation constant for the bacterial degradation of toluene is explicitly described for the strain *Pseudomonas putida* F1 by Reardon et al. (2000) and by Alagappan and Cowan (2004). Both studies are carried out with suspended cultures, however the half-saturation constant is a strain specific parameter, independent of the growth conditions. Unfortunately the $K_S^T$ values in the temperature range between 25 to 30 °C are in both references considerably different. They varied from $(13.8 \pm 0.9)$ g/m$^3$ to $(3 \pm 1)$ g/m$^3$, respectively. Lu et al. (2004) listed a toluene half-saturation constant for a toluene degrading trickle-bed filter of only 0.6 g/m$^3$. In their models of toluene degrading membrane bioreactors Parvatiyar et al. (1996) and Ergas et al. (1999) used values as small as 0.06 and 0.02 g/m$^3$, respectively. The value applied in the present model is set to 3 g/m$^3$, as is stated in the study of Alagappan and Cowan (2004). That detailed study of the strain *P. putida* F1 also states the maximum growth rate besides the half-saturation constant for toluene among other parameters, which agreed very well with the $\mu_{\text{max}}$, measured for the biofilm bacteria consortia in the reactor, consisting of approximately 40% of *P. putida* F1.
The oxygen half-saturation coefficient is a parameter which is difficult to determine exactly, it is therefore seldom stated in literature. Alagappan and Cowan (2004) specified the half-saturation coefficient for the strain \(P. \text{putida} \) F1 to be approximately \((1.1 \pm 0.47) \text{s/m}^3\). Zarook et al. (1997) measured an oxygen half-saturation constant of \(0.26 \text{s/m}^3\) for a biofilter inoculated with a mixed culture. Shaler and Klecka (1986) specified a half-saturation constant of \(1.2 \text{s/m}^3\) for growth on 2,4-dichlorophenoxyacetic acid and assumed, based on the results, that dissolved oxygen concentrations below \(1 \text{s/m}^3\) may be rate limiting for biodegradation of chlorinated aromatic compounds. This cognition is an indication that the applied value in the model, \(1 \text{s/m}^3\) with an arbitrarily chosen range of \(0.1 \text{s/m}^3\) to \(5 \text{s/m}^3\) for the parameter study, is the appropriate order of magnitude.

5.2.5.6 Yield coefficients

The yield coefficients are calculated based on measurements taken from the reactor.

\(Y_{\text{XT}}\) The apparent biomass to toluene growth yield amounts to \((0.31 \pm 0.15) \text{s/g} \) (dry biomass produced per mass toluene consumed). It is the average of the distinct yields for the nine steady state working points.

\(Y_{\text{XO}_2}\) The biomass to oxygen yield coefficient is calculated according to the stoichiometric equation stated in Chapter (4.4.11.2). This value expresses the apparent yield. The amount of oxygen consumed for maintenance is included. It is \((0.129 \pm 0.042) \text{s/g} \) (dry biomass produced per mass of oxygen consumed).

5.2.5.7 Maximum growth rate

\(\mu_{\text{max}}\) The maximum specific growth rate on toluene is measured for the mixed bacterial biofilm culture harvested 93 days after inoculation (see Chapter 3.9.6). This sample is composed of approximately 30%
5.2. MODELEQUATIONS

\textit{P. putida} F1 and 10\% \textit{R. globerulus} PWD1, while different toluene degraders constitute the remaining fraction. The measured value, used as the maximum specific growth rate in the model, is \((0.5 \pm 0.16) \, \text{h}^{-1}\). It is in good agreement with the value stated by Alagappan and Cowan (2004) of 0.42, measured for a pure culture of \textit{P. putida} F1 growing on toluene but at a temperature of only 20°C.

5.2.5.8 Bacterial mass

\(x^m\) The concentration of suspended dry biomass in the nutrient medium is estimated from optical density measurements of the culture medium performed at a wavelength of 600 nm (OD\(_{600}\)). The averaged OD\(_{600}\) amounted to 0.01. The optical density is converted to a biomass concentration using the standard correlation estimating that an OD\(_{600}\) of 1 equals \(10^9 \text{bacteria/ml}\). The geometric form of a bacterium is assumed to be a cylinder with a diameter of 1 \(\mu\text{m}\) and a length of 3 \(\mu\text{m}\). The density of the wet bacterial mass is assumed to be \(10^6 \text{g/m}^3\) and the dry mass is set to a fraction of 30\% (Cayley et al., 1991). The used biomass concentration in the model is 70 \(\text{g/m}^3\). The possible interval for the biomass concentration ranges from 14 to 160 \(\text{g/m}^3\). This interval results from varying the length of the assumed cylinder for the bacterial volume from 2 \(\mu\text{m}\) to 4 \(\mu\text{m}\) and by applying the measured OD\(_{600}\) range from 0.03 to 0.17.

\(M^b\) The total actively toluene degrading bacterial mass employed in the model is estimated from plate counts of resuspended biofilm (see Section 4.4.6.1 for the plate count results). This number of CFU is calculated to a dry bacterial mass applying the same procedure as explained for the suspended dry biomass; with the additional constraint that toluene degraders only constitute 40\% of this mass (see Section 4.4.6.2). The result of the sampled biofilm area has to be extrapolated to the total biofilm surface. A possible range of 0.05 to 1 g of total dry, active biomass in the reactor has to be specified. The model is calculated with 0.4 g dry, active biomass in the biofilm.
5.2.5.9 Bacteria distribution

The total dry bacterial mass is distributed along the height of the biofilm according to the green fluorescent intensity $I_{\text{green}}$ measured in the course of the CFLSM experiments (see Section 4.4.7). A double exponential curve is fitted to the measured data and the total bacterial mass $m_{\text{b}}$ is distributed along the the biofilm thickness $h_{\text{b}}$ according to the determined equation.

$P$ The number of points used in the non homogeneous grid along the $z$-direction in the biofilm is set to 50.

5.3 Verification

The verification of correct manual discretization of the spatial variable in the gas and biofilm is addressed in the following section.

The verification of the model describing the biological waste gas treatment system is done on the basis of the gas phase. Figure 5.2 shows the analytical and numerical solution for the differential equation of the toluene concentration in the gas phase along the length of the absorption membrane module. The numerical solution is plotted as a function of the grid points in the homogeneously spaced gas phase. The numerical solution overlays the analytical solution beyond approximately 20 grid points. Therefore the model is solved by use of 50 grid points to save calculation time but to calculate sufficient exactly.

The biofilm, the second phase for which the spatial variable is manually discretized, is also numerically solved by use of 50 grid points. The influence of the number of grid points on the course of the concentration along the distance in the biofilm is shown in Figure 5.3. For a number of grid points greater than 20 points the calculated profiles overlay. The grid in the biofilm is of a non-homogeneous form. This fact can easily be seen by the decreased spacing between the grid nodes towards the membrane.
5.4 Validation

5.4.1 Comparison of the modeled to the measured data

The model is validated by comparing the modeled data to selected parameters measured within or at the outlets of the reactor. The key parameter which actually accounts for the reactor performance is the toluene concentration in the gas phase at the outlet. The modeled data for the toluene outlet gas concentration are in good agreement with the measured values (see Figure 5.4). This is especially the case for the steady-state run conditions of the reactor at higher inlet gas concentrations (2.5 g/m³). At lower concentrations the modeled data underestimate the measured data. One reason for this fact might be, that the measurements are not as precise as the error bars may present, as indicated by the surface elimination capacity (see Figure 5.5). The measured and
Figure 5.3: Analysis of the necessary minimum number of grid points for the manual discretization of the biofilm.

Figure 5.4: The measured concentration of toluene in the gas phase at the outlet of the reactor (void markers) is compared to the modeled data (solid markers). The error bars indicate the total uncertainties. The modeled data are in agreement within the calculated total uncertainties.
The total uncertainty for the toluene gas outlet concentration only takes into account the random error of the measurements and the systematic error of the measuring device (FID). However, the outlet concentration is also influenced by the inlet gas concentration which is subjected to a large systematic error. The surface elimination capacity includes both uncertainties; from the loading unit and the outlet gas stream.

**Figure 5.5:** The surface elimination capacity, shown as a function of the surface load. The solid markers indicate the modeled data.

A further informative and crucial parameter at the outlet of the reactor which decides about the realistic operation of the model is the dissolved oxygen concentration in the bleed stream of the culture medium (Figure 5.6). The model is not able to exactly match the experimentally determined dissolved oxygen concentration values. However, it predicts the oxygen concentrations to be considerably different from zero for the correct steady-state working conditions. The deflection of the exact values can have several reasons. The most reliable might be the disregard of all aerobic active bacteria in the reactor which are not able to degrade toluene, amounting to approximately 60% of the total number of aerobic, culturable cells in the reactor (see Section 4.4.6.3). These bacteria grow on the intermediate products from the toluene degraders as well as on the produced EPS. This growth uses oxygen, but this consumption
is not included in the model. The metabolic activities of these strains as well as the characteristics of the available carbon source are not qualified. Parameters measured within the reactor which provide insightful information about the performance of the model are the toluene concentration of the absorbent as well as the dissolved oxygen profile in the biofilm. The model constantly underestimates the measured toluene concentration in the silicone oil (see Figure 5.7). One possible explanation for this deviation is that the mass transfer coefficient $k_{ov}^aT$ might be incorrect. In fact, the measurements of the mass transfer coefficient carried out in a preliminary test shows a considerable error of ±30%. Additionally, the flow profile of the silicone oil has to be changed from the preliminary experiment to the real set-up. In the preliminary experiment the flow of the absorbent is directed by a meander along the membrane. The superficial velocity is $0.25 \text{ m/s}$ corresponding to a $Re$-number of 0.2. In the reactor the absorbent is conducted by static mixers along the membrane. The superficial velocity – of lesser importance due to the static mixers – is approximately $2.5 \cdot 10^{-3} \text{ m/s}$. However, although this changed flow pattern may affect the boundary layer and with that the mass transfer coefficient, the model is not sensitive to a change of $k_{ov}^aT$.

![Figure 5.6: The dissolved oxygen concentration is shown as a function of surface load.](image)
5.4. VALIDATION

by the mentioned ±30%. The concentration of toluene in the absorbent changes by merely 2.7 g/m³. Therefore the answer to the question why the model does not predict the toluene concentration more accurately must be more complex and cannot be answered definitely.

Figure 5.8 shows three individually measured and the modeled dissolved oxygen concentration profiles. The model fits the measured data poorly. The equilibrium concentration on the membrane in the biofilm (solid circle) is in the same range as the experimentally determined concentration values. The model predicts a decrease to zero of dissolved oxygen in half of the measured distance. It is interesting to note that already the modeled concentration at the first grid node decreased from the equilibrium concentration on the membrane by more than 70%, although the thickness of this first section is manually set to only 1 · 10⁻⁹ m. The development of the modeled and the measured profiles are different. The measured ones show an interesting convex concave course with an inflection point in the range of 60 μm. This different profile course as well as the slower measured concentration decrease in the first few micrometers lead to several conclusions: the biofilm is not a homogeneous, pore free body featuring constant diffusion coefficients as assumed in the model.
Figure 5.8: Three independently measured dissolved oxygen profiles in the biofilm, taken in the course of the steady-state working point number one, are shown as a function of penetration depth. The solid line designates the related modeled oxygen profile; the solid maker stands for the equilibrium oxygen concentration with the absorbent in the aqueous phase on the membrane.

and secondly there must be a diffusion limiting layer in the biofilm close to the membrane. In fact with additional analysis of the ConA-reactive extracellular polysaccharides in the biofilm matrix (see Section 4.4.8) it can be shown that the concentration of these EPS seem to increase with decreasing distance to the membrane. Therefore, from graph 5.8 it can be concluded that for a more accurate model describing the biofilm, the EPS and their spatial distribution as well as the apparent diffusion coefficients as a function of the position in the biofilm have to be enclosed in the model.

5.4.2 Can diffusion in the gas phase be neglected?

5.9 shows the comparison between solutions of the differential equations for the toluene gas concentration, one derived including diffusion (Equation 5.5) along with convection the other considering only convection.
5.5. MODEL RESULTS

5.5.1 Sensitivity analysis

The sensitivity analysis is one reason why a model describing the reactor set-up is developed. The results are shown in diagrams of the type where the value of the change of a key parameter is plotted on the ordinate. It is important to note that the range of the ordinate varies for different sensitivity analyses. The abscissa shows a parameter multiplier, a factor by which the parameter in question is varied. This variation range is adapted from the error analysis for the experimentally determined parameters. For the literature derived parameters the range is limited by the minimum and maximum stated values. All parameters and their associated differences are discussed in Chapter 5.2.5.
The sensitivity analyses are only shown for the selected key parameter, the toluene gas concentration (except for the sensitivity of the oxygen diffusion coefficient in the biofilm). The analyses are calculated for the basic steady-state working point number four featuring a toluene inlet flow rate of 0.3 g/h and gas flow rate of 1 l/min. The resulting steady-state toluene gas outlet concentration for this working point, used as the reference for all sensitivity plots, is calculated to be 2.52 g/m³. It is assumed to be reached after 1000 h (it does not change by more than 0.02% during the last calculated 100 h). The negligence of all other important parameters requires proof that these change along the same pattern, together with the selected key parameter. This evidence is supplied by figures 5.10 and 5.11. Both plots show the sensitivity analysis for the microbiological parameters. One is done for the gas outlet concentration while the other for the toluene concentration in the culture medium. The curves shown in figures 5.10 and 5.11 follow the exact same pattern. The maximum growth rate is of major influence. The curves follow the same order and the crossings lie at the same positions. Even the relative deviations from the respective values are in the same range, calculated for the central operating condition. $\mu_{\text{max}}$ deviates by

![Figure 5.10: The sensitivity plot of the maximum growth rate and the half-saturation constants.](image)

$\mu_{\text{max}}$ deviates by
30% at a parameter multiplying factor of 0.7 for the gas as well as for the aqueous concentration. The model is highly sensitive to a decrease in the maximum growth rate, while an increase within the calculated uncertainty does not influence the key parameters by more than 10%. The influence of the half-saturation constants is not to be neglected either. Especially for the $K_S^T$-value there is a wide documented range, from a minimum value of $3 \text{s/m}^3$ up to $14 \text{s/m}^3$, which influences the toluene concentrations by more than 10%. From this sensitivity analysis of the microbiological parameters it has to be concluded, that these parameters are of great importance and that they have to be determined as accurately as possible for the conditions of the existing system. The apparent maximum growth rate can be experimentally determined to a sufficiently reliable value. However, the half-saturation constants can not be measured in the course of this thesis and they have to be taken over from literature data.

The sensitivity analysis of the biofilm model parameters is shown in two graphs. The sensitivity of the biofilm thickness $h^b$, of the total dry biomass in the reactor $M^b$, and of the suspended (dry) biomass concentration in the culture liquid $x^m$, is shown in Figure 5.12. The influence of
the yield coefficients is shown in Figure 5.13. The absolute biomass em-
bedded in the biofilm of the reactor is of significant influence, especially
for values lower than the one applied in the model. The large influence
of \( M_{\text{bot}}^b \) is unfavorable in the respect that this parameter could only be
measured to an approximate value. This is due to three reasons: first
taking a sample of an accurate area of a biofilm growing on a membrane
installed in a reactor, is very difficult. Second, the procedure of using
plate counts to obtain the number of culturable cells is known to be sub-
ject to large uncertainties. This is especially the case for biofilm samples
which are difficult to resuspend to give a homogeneous suspension of sin-
gle bacteria. The third reason is the calculation of a dry bacterial mass
from a number of cell forming units. This calculation is based on the
geometrical form and the size of the toluene degrading bacteria as well as
on the amount of water. The bacterial mass has, within the calculated
range, a maximum influence of 25 % on the toluene gas outlet concen-
tration for the smallest possible value. The biomass is distributed according
to the measured green-intensity over the height of the biofilm; the ab-
solute biomass thickness is therefore of no influence on the degradation
performance in the analyzed thickness range. The suspended biomass
concentration in the culture liquid is not of significance, since there is

Figure 5.12: The sensitivity plot of the biomass parameters.
hardly any dissolved oxygen nor any toluene available in the nutrient medium for bacterial growth or degradation.

Figure 5.13 shows the sensitivity of the used biomass yield coefficients on the gas outlet concentration of toluene. The model is extremely sensitive to both yield coefficients. For the investigated parameter multiplier range, $Y_{XT}$ shows a maximum influence of more than 60% on the toluene outlet gas concentration, and $Y_{XO_2}$ a maximum influence of more than 40%. This high sensitivity is very unfavorable because the uncertainty ranges for both parameters are large. As discussed in Section 4.4.10.2, the broad range is mainly due to the lack of an exact measurement of the EPS concentration in the culture medium. However, it has to be taken into account that the range of $(50 \pm 30) \text{g/m}^3$ is a very conservative estimation, which is chosen to include all possible concentration values, but which is therefore likely to be undercut.

The sensitivity plot for the diffusion coefficients in the biofilm is shown in Figure 5.14. The model is not sensitive to changes of the diffusion coefficient for toluene in the biofilm. However, the oxygen diffusion coefficient in the biofilm is of special significance again for parameter factors less than one. The diffusion coefficient is varied from 9% to 75% of the diffusion coefficient in water at 28°C featuring the salinity of M9.
Figure 5.14: The sensitivity plot for the diffusion coefficients in the biofilm.

For the smallest diffusion coefficient reported in toluene degrading *Pseudomonas* biofilms (9%), at the parameter multiplier of 0.24, the sensitivity analysis shows a deviation of the toluene outlet concentration of 12%. Notably, the model is more sensitive to a decrease of the parameter in question than to an increase.

The oxygen diffusion coefficient is an interesting parameter due to the fact that it has to influence the oxygen profile in the biofilm. This profile is experimentally determined but not accurately predicted by the model (see Figure 5.8). Therefore it is noteworthy to check whether a different diffusion coefficient would lead to a dissolved oxygen concentration in good agreement with the experimental results. Figure 5.15 shows the dissolved oxygen profiles as a function of the distance from the membrane as well as of the oxygen diffusion coefficient. The plot confirms the expected trend, namely a more rapidly decreasing profile for a higher diffusion coefficient. The graph also shows that the oxygen concentration in the first slice is higher for lower diffusion coefficients. This course is understandable, since the concentration of oxygen changes only by diffusive transport besides the biological degradation. Comparing figures 5.15 and 5.8 shows, that a variation of the oxygen diffusion coefficient within the reasonable range does not approximate the measured profiles.
Figure 5.15: The profile of dissolved oxygen concentration in the biofilm as a function of the oxygen diffusion coefficient. The diffusion coefficient is varied in the range of 9% to 75% of the coefficient in water. The line with circle marks denotes the standard diffusion coefficient incorporated in the model (40% of the diffusion coefficient in water).

Figure 5.16 shows the influence of varying partition coefficients on the model results. The partition coefficient for oxygen between gas and absorbent shows the strongest influence on the toluene gas outlet concentration. Although at first glance $H_{gaO_2}$ seems to have a significant influence this is not the case, the gas outlet concentration does not change by more than 10%. The partition coefficients between mineral medium and absorbent $H_{maSi}$ are varied over a broader range than the partition coefficients between gas and absorbent $H_{gaSi}$, but they are of minor influence, changing $c_{gT}$ only by 3%. Figure 5.17 shows the results of the sensitivity analysis of the mass transfer coefficients. The graph clearly states that the model only depends on the oxygen mass transfer from the gas to the absorbent, while the variations of all other mass transfer coefficients can be neglected. Although at first glance one might get the impression, that $k_{ovO_2}$ is the key parameter to the model, this is not the case. Looking at the absolute change of the key parameter, the variation
of $k_{ov}^{aO_2}$ in the calculated uncertainty range influences $c_a^{mT}$ by only 8%. However, the small calculated range – 90% to 105% of the value used in the model – needs critical reflection. As stated earlier (see Section 3.4.3)
5.5. MODEL RESULTS

the mass transfer coefficient for oxygen across the dense PDMS membrane from bulk gas to bulk absorbent is estimated based on the film theory. The measured oxygen diffusion coefficient in the membrane and an estimated mass transfer coefficient from the membrane to the bulk of the absorbent are used. The diffusion coefficient as well as the the mass transfer coefficient across the absorbent boundary are reasonably accurate. However, the exact influence of the static mixers, used as support for the membrane, on the flow profile is unknown. Computational fluid dynamics modeling of one segment of a static mixer showed that the velocity of the silicone oil is not constant. There are stagnant regions in the corners of these compartments, while the central zones are highly mixed. Therefore the application of a $Sh$-correlation derived for a flat channel in a static mixed flow is likely to increase the uncertainty range. Such an increase of $k_{\text{ov}}^{aO_2}$ to $\pm 30\%$ as well as for $k_{\text{ov}}^{aT}$ would increase the absolute change of the toluene gas outlet concentration by approximately $\pm 25\%$. This increases the influence of $k_{\text{ov}}^{aO_2}$ up to the same range as the parameters $\mu_{\text{max}}$ or $K_S^{O_2}$.

These sensitivity analyses lead to the conclusion that the parameters featuring the biggest influence on the toluene gas outlet concentration are a decreasing total dry bacterial mass in the biofilm, as well as a decreasing maximum specific growth rate. However, it cannot be stated that either of these parameters is the limiting factor of the degradation rate of the model system. The mass transfer coefficient for oxygen across the membrane from the gas to the absorbent phase as well as the partition coefficient for oxygen between the gas and the absorbent are also of significance. These become even more important in the case of an underestimation of the error ranges for the latter mentioned physical parameters for oxygen. Therefore, no final conclusion can be drawn on the limiting factor of this model based on this sensitivity analysis.

5.5.2 Time to reach steady state

The reactor is run under consecutively adjusted steady-state operating conditions depending on the mass flow rate of toluene and on the gas flow rate. One central question when operating a reactor at a multitude of steady-state conditions is how long the system needs to reach
steady-state. In the experimental analysis these time periods are set individually for each step change, based on the uncertainty analysis. The average period is seven days until steady-state is achieved. Figure 5.18 shows the modeled toluene outlet gas concentration changing with time. Two curves are shown, the upper line describes the course of the outlet concentration for an inlet concentration change from 5 g/m$^3$ to 2.5 g/m$^3$, the lower line indicates a change from 2.5 g/m$^3$ to 1.25 g/m$^3$. The concentration step is accomplished at 200 h. The graph shows that for smaller concentrations the seven day period (marked in the graph by the dotted line) is sufficient; the deviation of the outlet concentration 170 hours after the change amounts to 0.3% compared to the new final steady state (assumed to be reached 1’000 h after the step-change). However, by increasing the inlet concentrations, and with that the absolute concentration difference, the time to reach steady-state is extended. The toluene outlet concentration seven days after the change, deviates from the steady-state concentration by 7% (assumed to be reached again 1’000 h after the concentration change). From this result it has to be
concluded that especially for the concentration changes above 5 g/m³ the waiting period between steady-state experiments are probably not sufficiently long to reach steady-state. Measurements carried out in the reactor which is not at steady-state would lead to an underestimation of the calculated uncertainties.

5.5.3 Maximum degradation rate

The model can also be used for calculating the maximum degradation capacity of the reactor. Figure 5.19 shows the experimentally determined surface elimination capacities in comparison to the modeled ones.

![Figure 5.19: Modeled surface elimination capacities for higher surface loads are plotted along with the measured data. The solid markers represent the modeled values.](image)

Surface elimination capacities for higher surface loads (up to five times more than the largest measured one) are modeled to categorize the maximum elimination capacity, which is shown on the graph as amounting to 0.55 g/m²h⁻¹. It is interesting to note that for increasing surface loads the surface elimination capacities are not dependent on the gas flow rate anymore, the modeled data points overlay each other. This behavior is
explicable by the fact, that the maximum surface elimination capacity is a microbiological constant, independent of the mass transfer rate beyond a certain value. This value is approximately reached for surface loads higher than \(3 \text{g/m}^2\text{h}\). The elimination capacities acquired for the steady-state operating conditions with a surface load of \(3.29 \text{g/m}^2\text{h}\) are therefore assumed to be in the reaction limited regime.

These analyses lead to the conclusion that the reactor – according to this model – is limited by mass transfer for surface loads lower than \(3 \text{g/m}^2\text{h}\), and is reaction limited for higher surface loads. The mass transfer comprises the transport of toluene and oxygen from the gas phase to the bacteria in the aqueous phase. In the reaction limited regime the degradation capacity of the reactor is reached and the remaining toluene leaves the reactor via the off-gas or the bleed stream of the culture medium.

5.5.4 Concentration pattern of toluene in the absorption module

The model is also used to determine the maximum gas flow rate appropriate for the existing absorption module. The key parameter is the residence time of the gas within the module to reach equilibrium with the absorbent. This residence time depends on the geometric dimensions, mainly the length, of the absorption module. The residence time amounts to 54.8 s for a gas flow rate of \(1 \text{/min}\) and decreases to 27.4 and 13.7 s for 2 and \(4 \text{/min}\), respectively. These times are calculated based on the assumption of a superficial gas velocity. Figure 5.20 shows an analysis of three consecutive gas flow rates for a constant toluene mass flow of \(0.3 \text{g/h}\). This procedure is adopted from the existing set-up. The graph shows decreasing inlet gas concentrations of \(5 \text{g/m}^3\), \(2.5 \text{g/m}^3\) and \(1.25 \text{g/m}^3\) for increasing gas flow rates. The toluene gas concentration changes with position along the membrane. For the steady-state condition featuring a gas flow rate of \(1 \text{/min}\), it is obvious that the gas and the absorbent reach equilibrium already in the first third of the module. The gas concentration at this position in the module (0.23 m) reached 97.2% of the value of the departing gas. The course of the concentration profile for a gas flow rate of \(2 \text{/min}\) shows an analogous pattern. After passing 0.47 m of the absorption module the toluene gas concentration deviates
5.5. MODEL RESULTS

Figure 5.20: The toluene gas concentration is shown as a function of the position in the absorption module.

from the outlet concentration by only 2%. Based on these two model runs it can be concluded, that to reach more than 95% of the final gas concentration the absorption module is built sufficiently long; secondly, the overflown distance in the absorption module approximately doubles by doubling the gas flow rate to reach steady-state. The concentration profile for the gas flow rate of 4 l/min seems to follow a similar decrease along the membrane. However, it can not be stated that the module is long enough to establish equilibrium to the same extent. Therefore the first spatial derivative of the toluene gas concentration is calculated (see Figure 5.21).

This plot confirms, that for a gas flow rate of 4 l/min the absorption module is also of sufficient length. The first derivative of this gas flow rate reaches a similar small value close to zero as is the case for the derivatives of the other flow rates. In conclusion, these model results show that the existing absorption module with a length of 0.7 m is adequate to reach equilibrium for gas flow rates up 4 l/min.
5.5.5 Influence of the non-homogeneous bacterial mass distribution in the biofilm

One key feature of the model is the distribution of the actively toluene degrading biomass according to the course of the measured green-intensity along the thickness of the biofilm. Which raises the question whether this distribution is of any importance for the model results. The influence of this biomass distribution on the surface elimination capacity is shown in graph 5.22 and the influence on the dissolved oxygen concentration in the biofilm, a second important parameter in this context, is shown in graph 5.23.

The influence on the surface elimination capacity is large. The modeled data underestimate the measured values. The graph shows that the maximum elimination capacity would only be in the range of 0.25 instead of 0.55 g/m²h. Assuming a homogeneous distribution, this underestimation can be reduced by increasing the biomass. However, even with a tenfold mass of active bacteria – although this value is not in agreement with the calculated error range – the model would still underestimate the experimentally determined data (not shown). Figure 5.23 shows the
two resulting courses of the oxygen concentration in the biofilm; one, the biomass is distributed homogeneously throughout the biofilm and two, it is spread according to the green intensity measurements, localizing the living bacteria. A model using a homogeneous biomass distribution would not describe the measured profiles either. As expected, the dissolved oxygen concentration decreases slower for homogenously distributed bacteria. There is less biomass consuming oxygen in the bottom layer of the biofilm, which causes more oxygen to diffuse deeper into the film. The equilibrium aqueous oxygen concentration on the membrane is also higher, overestimating the experimental data. This behavior is explicable by the same argumentation. Fewer bacteria in the bottom layer of the film use less oxygen, reducing the driving concentration gradient and leading to a higher oxygen concentration in the absorbent. This investigation can be summarized to state that the biomass distribution is a good, novel base for more accurate biofilm degradation models. However, also the approach using the measured green-intensity
Figure 5.23: Two modeled dissolved oxygen concentration profiles in the biofilm are shown, one for a homogeneous biomass distribution, the other for the distribution according to the green fluorescence intensity. The solid black triangles denote the oxygen concentration in equilibrium with the absorbent on the membrane in the biofilm (∇ non-homogeneous, △ homogeneous). For comparison reasons three experimentally determined oxygen profile are plotted.

alone as foundation for the biomass distribution is not sufficient for a precise prediction of the oxygen profile. A biofilm is too complex to assume it as a homogeneous, dense, pore free body, featuring constant diffusion coefficients. Therefore, the active biomass distribution along the biofilm height is only one element of future exact biofilm models.

5.6 Towards an optimized set-up

5.6.1 Limits of the current set-up

The ratio of the biofilm area to gas flow rate, which is chosen for our set-up, is based on a previous study (Vinage, 2002), carried out at the Institute of Process Engineering (ETHZ). The ratio of the areas provided
for absorption and for biofilm growth, could only be estimated based on an early model. This model lacked measured, accurate parameters, to determine the appropriate ratio. The question arises, whether the chosen ratio of $A_{\text{biof}} / A_{\text{abs}}$, 1.5, is correct.

Figure 5.24 shows the toluene gas concentration at the outlet of the reactor as a function of the ratio between the absorption and the biofilm area. It is important to note that the analysis is carried out for a constant absorption area. The data are modeled using a gas flow rate of $1 \ell/min$ and a constant inlet concentration of $5 \text{s}/m^3$. This analysis shows that

![Toluene outlet gas concentration](image)

**Figure 5.24:** The toluene outlet gas concentration is shown as a function of the ratio of the biofilm area to the absorption area. The absorption area is kept constant at $0.18 \text{m}^2$. The gas flow rate is set to $1 \ell/min$, the inlet concentration to $5 \text{s}/m^3$. The dotted line represents the ratio, which is used in the present set-up.

with a different area ratio a lower outlet concentration of toluene could be reached. In that sense the system still has some reserve. An enlargement of the area ratio to five, for example, would lower the outlet concentration by more than 35%. This would correspond to a removal efficiency of 66%. Considering the additional investment costs an optimum between technical feasibility and costs must be evaluated.

Figure 5.24 shows that an enlargement of the ratio is only appropriate
up to certain value, since the toluene outlet concentration approaches asymptotically a constant value of approximately 1 g/m³. The fact that this value is different from zero, is an important property of the present system using an absorption step. In the current system all the VOCs must pass the absorbent prior to biological degradation. This means that for the transport of VOCs through the absorbent to the biofilm, VOCs have to be present in the absorbent. In an absorber the off-gas is in equilibrium with the absorbent. Therefore, the toluene outlet gas concentration reaches a minimum of 1 g/m³.

5.6.2 Amelioration of the proposed waste-gas treatment system

Two possible solutions, using absorption, are considered to decrease the toluene concentration in the outlet gas, either using a different absorbent with a more favorable gas-absorbent partition coefficient or planning a multistage set-up.

A smaller partition coefficient would lower the gas concentration, which is in equilibrium with the absorbent. However, it has to be considered that VOCs, which are targeted with waste gas treatment systems using organic phases, are poorly water soluble. These are VOCs with low gas-water partition coefficients, which is linked to the gas-absorbent and the absorbent-water coefficient (see Chapter 4.1.2). Therefore, a very small gas-absorbent coefficient also means a small absorbent-water coefficient for poorly water soluble VOCs. This lowers the mass transfer rate to the biofilm and is therefore unfavorable.

The second solution is to design a multistage set-up. The idea is to introduce a buffering system for the first stage, while the following stages do not need to be buffered, since the off-gas from the first stage is virtually constant. Figure 5.25 shows the simulation results of a fluctuating inlet gas concentration for a gas flow rate of 1 l/min. The model is calculated with the same ratio of absorption to biofilm area as it is used in the real set-up. The outlet gas concentration is virtually constant, in spite of the large fluctuations of the inlet loads. The introduced absorption buffers the load fluctuations. The off-gas with a constant load of VOCs
can be cleaned using one (or more) bioreactor(s) without a buffering system. In this theoretical approach the following stages are modeled as conventional membrane bioreactors, where the membrane separates the gas from the aqueous phase. The membrane area of the biofilm is equal to the one in the existing set-up. Figure 5.26 shows the modeled toluene concentration in the off-gas of a multistage system. The model is evaluated for the above described course of a fluctuating toluene inlet gas concentration. This concentration profile is shown in Figure 5.26 with a data point at 5 g/m³. The resulting outlet gas concentration is used as the inlet concentration for the following non-buffered membrane bioreactor. The maximum fluctuations in concentration of the gas stream leaving the first buffered stage are marked with an error bar. The Swiss legal limit for toluene, 0.1 g/m³, is almost reached with the addition of a second, non-buffered bioreactor. This outlet concentration corresponds to a removal efficiency of 95%. To reach the target limit, using the dimensions of the existing set-up, a third stage would be necessary. However, it has to be taken into account that neither the absolute areas in comparison to the gas flow rate nor the ratio of the absorption to the biofilm area in the first stage are optimized. It can be concluded
Figure 5.26: The toluene outlet concentrations for three membrane bioreactors in series are shown. The first stage is modeled with a buffer, while the other two are not.

that with an optimized design of the distinct membrane modules two stages would be sufficient to reach the target limit. This analysis shows that a proper, optimized multistage system would allow to reach the stated objective.
Chapter 6

Summary and conclusion

6.1 Summary of the experimental and mathematical investigations

6.1.1 Experimental set-up

An experimental biological waste gas treatment set-up was constructed and successfully run for a period of 162 days. The set-up consisted of an absorption and a biological degradation step. In both unit operations flat membranes were applied to separate the two respective fluids. The same, novel, thin, dense, reinforced PDMS membranes were used for both membrane processes, kept in place by pressing them against a support structure by a small transmembrane pressure difference. The supporting surface was made of static mixers, in order to reduce the thickness of the hydrodynamic boundary layer on the membrane and thereby reduce the mass transfer resistance. The absorbent, as well as the culture liquid, were circulated in loops. The absorbent was run from a storage tank to the absorption-module, to the bio-module, and back...
to the tank. The culture liquid was carried in a loop to flow over the biofilm with a known fluid velocity.

6.1.2 Treatment of hydrophobic VOC

The model VOC was toluene. It is only slightly water soluble and it features a high air-water partition coefficient, which hampers the bacterial degradation in conventional bioreactors. With the present reactor, toluene was removed from the gas phase and readily degraded by an aerobic bacterial biofilm, growing on the membrane. The degradation capacity was shown to be mass transfer limited for surface loads smaller than approximately $4 \, \text{g/m}^2\text{h}$. The maximum surface elimination capacity, based on the biofilm area, amounted to $0.6 \, \text{g/m}^2\text{h}$.

The removal capacity of the plant for this hydrophobic VOC was easily comparable to data previously reported on toluene degrading membrane bioreactors. This, in spite of additional mass transfer resistances, introduced by the second membrane and the absorbent.

6.1.3 Buffering of VOC and oxygen

The selected absorbent was silicone oil. It was chosen due to its favorable partition coefficients as well as its high solvation capacity for toluene and oxygen. Experiments conducted with fluctuating inlet loads showed excellent buffering capabilities of the reactor, both substrates were readily buffered in the absorbent. This enabled the removal of high peak loads from the waste gas and periods of VOC free gas could be bridged, hereby ensuring a constant feed rate of toluene and oxygen to the bacteria in the biofilm.

6.1.4 Control of biomass accumulation

The biofilm grew directly on the membrane, which separated the absorbent from the culture liquid. The thickness of the biofilm was controlled by erosion or sloughing. This biomass removal was achieved by
flowing the nutrient medium in a sufficiently high fluid velocity over the biofilm, to introduce shear stress to the biofilm surface. It was shown during the operation of the reactor that a superficial velocity of the culture medium of 0.06 m/s was sufficient to keep the biofilm thickness below 1 mm.

6.1.5 Biofilm characterization

The biofilm was analyzed with respect to the spatial distribution of the bacterial activity and the composition of the microbial consortium. The spatial information was obtained by acquiring oxygen concentration profiles along the biofilm thickness and by confocal laser scanning microscopy (CFLSM) analyses to gather information about the distribution of the ratio of living to dead bacteria in the biofilm. Both experimental procedures showed the same result; only the base film – the first 80 μm next to the membrane – is metabolically active. The remaining, approximately, 85% of the biofilm thickness was inactive. This activity profile in the biofilm was advantageous considering that the top part of the biofilm was cleared away by the shear stress introduced. The most active part of the biofilm was left unharmed.

The reactor was run under non-sterile conditions. It was inoculated with two toluene degrading bacterial strains *Pseudomonas putida* F1 and *Rhodococcus globerulus* PWD1. The fraction of the biofilm bacteria, able to metabolize toluene, decreased within the first 50 days after inoculation to a constant value of only 40%. The remaining 60% must have grown on degradation products of the toluene degraders, since there was no other C-source available. The toluene degraders were composed of a considerable fraction – between 40 and 70% – of bacteria other than *P. putida* F1 and *R. globerulus* PWD1. According to visual inspection of the bacterial colonies, grown on selective plates, there were 4 new strains present in the reactor. The strain *R. globerulus* virtually vanished, while *P. putida* made up the remaining fraction.
6.1.6 Modeling

The experimental set-up was modeled. The four phases, gas, absorbent, biofilm and nutrient medium were modeled in equilibrium at their boundaries. The membranes separating the phases from each other were not explicitly included in the model. They appear indirectly in the corresponding overall mass transfer coefficients. For each phase, differential equations of the toluene and oxygen concentration were derived. The active biomass was distributed along the biofilm thickness according to the CFLSM measurement results of the living cells. This distribution was an attempt to omit black-box modeling, however, it must be noted that the distribution of the active bacteria in the biofilm alone is not sufficient to accurately describe the oxygen concentration profile. The sensitivity analysis indicated that the toluene outlet gas concentration was strongly influenced by microbiological parameters such as the yield coefficients, the specific maximum growth rate or the mass of bacteria degrading toluene.

6.2 Proposal for an optimized reactor concept

The ratio of the absorption to the biofilm area could not be be taken into consideration during the design of the experimental set-up. A model based analysis showed that the biofilm area is too small in comparison to the used absorption area. A biofilm area five times larger would enhance the removal efficiency considerably.

However, since an absorption is inserted into the biodegradation process, the VOC concentration cannot fall below a certain value. Absorption is an equilibrium process, were the gas and liquid reach equilibrium. This signifies that in the case of VOCs buffered in the absorbent, there are VOCs present in the off-gas.

To reach high removal efficiencies and to meet emission standards a multistage set-up is proposed. Two or more membrane bioreactors are placed in a series, whereas only the first reactor is equipped with an absorption
unit. This configuration buffers fluctuating loads and reaches high removal capacities. To reduce the complexity of a multistage membrane bioreactor one option is to circulate the same culture liquid through all stages, since the experiments and the modeling showed very small toluene concentrations in the nutrient medium. Modeling of this multistage approach demonstrated that this kind of process can reach the Swiss legal limit in a three stage bioreactor, even though important parameters like the membrane areas were not optimized.
Appendix A

Experimental results
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<th>$\frac{v_{m/3}}{\theta_{c_0}}$</th>
<th>$\frac{v_{m/3}}{\theta_{c_0}}$</th>
<th>$\frac{v_{m/3}}{\theta_{c_0}}$</th>
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**Table A.1:** The experimental results for the gas phase of the nonequilibrium steady state operating conditions (OC).
Table A.2: The experimental results for the absorbent phase of the nine steady state operating conditions.

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<th>$c^{aO_2}$ % (air-sat)</th>
<th>$T^m$ °C</th>
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<tr>
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<td>655.9 ± 59.3</td>
<td>73.1 ± 1.6</td>
<td>26.9 ± 0.4</td>
</tr>
<tr>
<td>9</td>
<td>350.8 ± 4.0</td>
<td>73.1 ± 0.9</td>
<td>26.7 ± 0.3</td>
</tr>
</tbody>
</table>
### Table A.3: The Experimental Results for the Nutrient Medium Phase of the Immune System Operation

<table>
<thead>
<tr>
<th>Condition</th>
<th>OD at 600 nm</th>
<th>H2</th>
<th>H2O</th>
<th>(be)H2O</th>
<th>( % \text{ au/s} )</th>
<th>( \text{mL/mg} )</th>
<th>OC</th>
</tr>
</thead>
<tbody>
<tr>
<td>100.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>200.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>300.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>400.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>500.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Note: The values in the table represent measured data for different conditions.
Table A.4: The elemental composition of the biomass, which is filtered off from the culture medium, for the investigated steady state operating conditions.

<table>
<thead>
<tr>
<th>OC</th>
<th>C (%(w/w))</th>
<th>H (%(w/w))</th>
<th>N (%(w/w))</th>
<th>O (%(w/w))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.406</td>
<td>0.061</td>
<td>0.093</td>
<td>0.440</td>
</tr>
<tr>
<td>2</td>
<td>0.385</td>
<td>0.059</td>
<td>0.008</td>
<td>0.548</td>
</tr>
<tr>
<td>3</td>
<td>0.287</td>
<td>0.049</td>
<td>0.0543</td>
<td>0.609</td>
</tr>
<tr>
<td>4</td>
<td>0.319</td>
<td>0.051</td>
<td>0.064</td>
<td>0.565</td>
</tr>
<tr>
<td>5</td>
<td>0.280</td>
<td>0.048</td>
<td>0.050</td>
<td>0.622</td>
</tr>
</tbody>
</table>
Appendix B

Model parameter
Table B.1: The standard model parameters and their sources.

<table>
<thead>
<tr>
<th>Model parameter</th>
<th>Value</th>
<th>Source/reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V^g$</td>
<td>0.911</td>
<td>experimental set-up</td>
</tr>
<tr>
<td>$V_g$</td>
<td>1 l/min</td>
<td>experimental set-up</td>
</tr>
<tr>
<td>$A^g$</td>
<td>0.19 m$^2$</td>
<td>experimental set-up</td>
</tr>
<tr>
<td>$V^a$</td>
<td>71</td>
<td>experimental set-up</td>
</tr>
<tr>
<td>$A^a = A^g$</td>
<td>0.19 m$^2$</td>
<td>experimental set-up</td>
</tr>
<tr>
<td>$h^b$</td>
<td>1 mm</td>
<td>estimation</td>
</tr>
<tr>
<td>$A^b$</td>
<td>0.27 m$^2$</td>
<td>experimental set-up</td>
</tr>
<tr>
<td>$V^m$</td>
<td>10 l</td>
<td>experimental set-up</td>
</tr>
<tr>
<td>$V_m$</td>
<td>0.209 l/h</td>
<td>experimental set-up</td>
</tr>
<tr>
<td>$T^g$</td>
<td>25°C</td>
<td>experimental set-up</td>
</tr>
<tr>
<td>$T^a = T^m$</td>
<td>28°C</td>
<td>experimental set-up</td>
</tr>
<tr>
<td>$H^{gaT}$</td>
<td>$1.06 \cdot 10^{-3}$</td>
<td>measurement</td>
</tr>
<tr>
<td>$H^{gaO_2}$</td>
<td>5.04</td>
<td>measurement</td>
</tr>
<tr>
<td>$H^{maT}$</td>
<td>$3.6 \cdot 10^{-3}$</td>
<td>measurement</td>
</tr>
<tr>
<td>$H^{maO_2}$</td>
<td>0.12</td>
<td>measurement</td>
</tr>
<tr>
<td>$k^{aT}_{ov}$</td>
<td>$4.0 \cdot 10^{-3}$ m/h</td>
<td>measurement</td>
</tr>
<tr>
<td>$k^{aO_2}_{ov}$</td>
<td>$6.7 \cdot 10^{-3}$ m/h</td>
<td>measurement</td>
</tr>
<tr>
<td>$\beta^{mT}$</td>
<td>$1.3 \cdot 10^{-2}$ m/h</td>
<td>$Sh$-correlation</td>
</tr>
<tr>
<td>$\beta^{mO_2}$</td>
<td>$3.0 \cdot 10^{-2}$ m/h</td>
<td>$Sh$-correlation</td>
</tr>
<tr>
<td>$D^{bT}$</td>
<td>$3.9 \cdot 10^{-6}$ m$^2$/h</td>
<td>e.g. Ottengraf and Vandenoever (1983)</td>
</tr>
<tr>
<td>$D^{bO_2}$</td>
<td>$8.64 \cdot 10^{-6}$ m$^2$/h</td>
<td>Beyenal et al. (1998)</td>
</tr>
<tr>
<td>$c_{0}^{mO_2}$</td>
<td>7.36 g/m$^3$</td>
<td>Ramsing and Gundersen (2004)</td>
</tr>
<tr>
<td>$K^T_S$</td>
<td>3 g/m$^3$</td>
<td>Alagappan and Cowan (2004)</td>
</tr>
<tr>
<td>$K^{O_2}_S$</td>
<td>1 g/m$^3$</td>
<td>Alagappan and Cowan (2004)</td>
</tr>
<tr>
<td>$\mu_{max}$</td>
<td>0.50 l/h</td>
<td>measurement</td>
</tr>
<tr>
<td>$x^{m}$</td>
<td>70 g/m$^3$</td>
<td>experimental set-up</td>
</tr>
<tr>
<td>$M^b$</td>
<td>0.8 g</td>
<td>experimental set-up</td>
</tr>
</tbody>
</table>
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