Membrane bioreactor for waste gas treatment

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Membrane bioreactor for waste gas treatment

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STELLINGEN

1 Onderzoek aan membraanbioreactoren voor gasreiniging dient vooral gericht te zijn op de stabiliteit van biofilms.
Dit proefschrift.

2 De door Parvatiyar *et al.* uitgevoerde experimenten vormen geen bewijs voor het door hen gepostuleerde model, dat beschrijft dat trichlooretheen anaeroob wordt omgezet en de reactieproducten vervolgens in de aerobe zone worden afgebroken. Tijdens de experimenten kan ook uitsluitend aerobe afbraak of uitsluitend anaerobe afbraak hebben plaatsgevonden.
Parvatiyar *et al.* 1996. Treatment of trichloroethylene (TCE) in a membrane biofilter. Biotechnol. Bioeng. **50**: 57-64.

3 Bij het vergelijken van elektronenmicroscopische foto's is het van groot belang dat de foto's min of meer dezelfde vergroting hebben.
N.A. Sutton *et al.* 1994. A *comparison of conventional* SEM *techniques, low temperature* SEM and the electroscan wet scanning electron microscope to study the structure of a biofilm of *Streptococcus crista* CR3. J. Appl. Bact. **76**: 448-454.

4 Het modelleren van een nieuw biologisch proces zonder zelfs maar een poging tot validatie, leidt tot een model, dat geen afbeelding van de werkelijkheid is, maar een vlucht eruit.
Ilias, S. and K.A. Schimmel. 1995. Membrane bioreactor model for removal of organics from wastewater. J. Air Waste Manage. Assoc. **45**: 615-620.

5 Met "kan niet" en "weet niet" is men het snelst van een klus af.
Mijn oma Moe.

6 De wens om representatief gekleed te gaan, is funest voor het woon-werkverkeer per fiets.

7 Instanties die adressenbestanden aanleggen om alleen "gerichte" en "gewenste" reclame te versturen, gaan voorbij aan het feit dat die reclame ook ongewenst kan zijn, juist omdat die geadresseerd is en als inbreuk op de privacy kan worden beschouwd.

8 Het is een wijdverbreid misverstand, dat emmertjes voor groenafval alleen in de container geleegd kunnen worden als ze vol zijn.

9 Het ontbreken van deugdelijke lusjes aan theedoeken doet vermoeden dat de ontwerpers ervan zelf een vaatwasmachine hebben.

10 De begrippen "multidisciplinariteit" en "een samengeraapt zootje" liggen dicht bij elkaar.

11 Het verplicht stellen van een grotendeels vastgesteld onderwijsprogramma voor promovendi is in tegenspraak met de oorspronkelijke doelstelling van promotieonderzoek; opleiden tot zelfstandig wetenschapper.

12 De vertaling van het Engelse woord *layout* luidt in het Nederlands niet *uitleg*, maar opzet, opmaak of ontwerp. Toch heeft de *layout* van teksten meer invloed op de *uitleg* ervan dan de schrijvers vaak vermoeden.

Stellingen behorende bij het proefschrift
"Membrane bioreactor for waste gas treatment".

Martine Reij
14 februari 1997

Voorwoord

Voordat u grote of kleine stukken uit dit proefschrift leest, of er alleen maar in bladert, wil ik de aandacht vestigen op alle mensen die er een bijdrage aan hebben geleverd.

Op de eerste plaats wil ik Sybe Hartmans bedanken. Vele uren heeft hij meegedacht over proefjes en artikelen, vooral toen mijn werk niet zo vlotte. Heel erg bedankt voor de prettige samenwerking. Ook Jan de Bont wil ik hartelijk bedanken voor de begeleiding. Zijn kritische blik bracht vaak de hoofdlijnen van onderzoek en artikelen weer helder voor ogen. Van Sybe en Jan heb ik heel veel geleerd. Verder wil ik ook de begeleidingscommissie onder leiding van professor Heijnen van harte bedanken voor hun belangstelling en voor hun waardevolle adviezen.

Verschillende studenten hebben een bijdrage geleverd aan het onderzoek dat in dit boekje beschreven staat: Lida Nugteren-Davoodzadeh, Jasper Kieboom, Alja Bezoen, Marieke Bruins, Erik Hamann en Leonie Hüsken. Behalve de door hen behaalde resultaten, heb ik ook de samenwerking met hen zeer gewaardeerd.

Al mijn (ex-)collega's van de Sectie Industriële Microbiologie wil ik bedanken voor hun belangstelling en voor hun hulp in het lab en met de computer. Maar belangrijker was nog de gezelligheid op het lab, in de AIO-kamer en soms ver daarbuiten. Ik zal vooral de lab-uitjes en de vrijdagmiddagborrels missen. Maar jullie kunnen nu eindelijk ongestoord aan de borrelnoten....

De medewerkers van de werkplaats, de afdeling fotografie en de afdeling elektronica wil ik bedanken voor hun vakmanschap en hun hulpvaardigheid.

Lex heeft er vaak voor gezorgd dat ik niet te hard werkte en ook aandacht voor andere zaken dan werk had. Bedankt voor alle geduld en goede zorgen.

Hoewel mijn vader het verschijnen van dit boekje niet meer heeft mogen meemaken, wil ik hem en mijn moeder bedanken voor de kansen die ik van hen kreeg en voor hun nimmer aflatende belangstelling. Dit proefschrift draag ik aan hen op.

Martine

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CHAPTER 1

General introduction

Biological waste gas treatment

Many industrial activities result in the emission of organic pollutants into the air. These pollutants can cause odour nuisance or, in the worst case, endanger health. Along with the increasing public concern on environmental issues, reduction of air pollution has been pursued by emission prevention and off-gas treatment and emission guidelines have been formulated in the Netherlands in 1992 (24).

A relatively cheap technique for off-gas treatment is biological treatment, or biofiltration. Biological waste gas treatment is based on the ability of microorganisms to degrade various organic pollutants and to use these pollutants for growth. In this way pollutants are degraded into harmless products like CO_2, H_2O and new biomass (Figure 1). Biological treatment is especially suitable for the removal of relatively low concentrations of organics (< 1-5 g m^{-3}) (27).

From the early 1960s biofiltration has been used to control odour and stench resulting from e.g. sewage works, composting plants, food processing and chicken or pig farms (15). In the early 1980s the field of application was extended to many other volatile compounds that are easily biodegraded. Since then the number of installations for biological air purification has increased significantly (27).

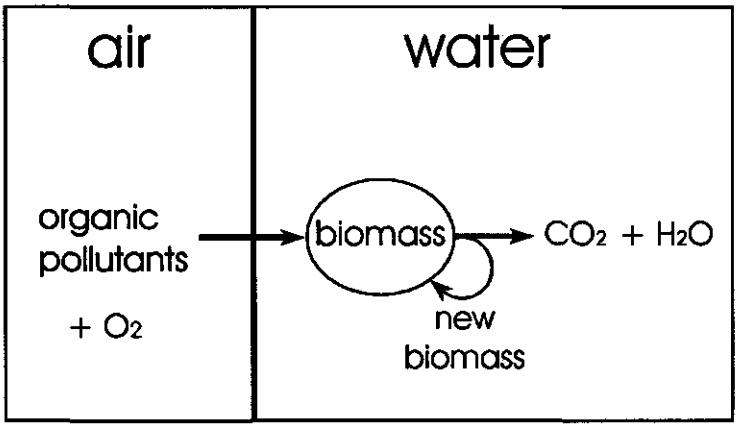

Figure 1. Biological oxidation of gaseous organics.

Bioreactors for waste gas treatment

The earliest type of bioreactor for waste gas treatment is the **biofilter**, containing compost, peat or soil and a coarse fraction to prevent high pressure drops (Figure 2A). The specific gas-liquid surface area of biofilters is with 300-1000 m^2 m^{-3} relatively large (20) and the mass transfer properties of biofilters are quite good. Biofilters have been used on an industrial scale to remove various pollutants from air, like alcohols, undefined odours, aromatics, esters (27) and have also been tested for the removal of aliphatics (18).

Treatment of gases containing chlorinated pollutants, sulphur compounds or ammonia results in the accumulation of chlorine, sulphate, or nitrate ions and causes acidification of the biofilter. Acidification can, to a certain extent, be prevented by the addition of e.g. lime, but large amounts of mineral end-products can not be neutralized nor removed from a biofilter (9, 14). To prevent dehydration of the biofilter, the waste gas has to be prehumidified. Nevertheless, moisture control in biofilters is difficult (18) and malfunctioning of biofilters is often due to problems with the humidity control (14).

A second type of bioreactor is the **trickle-bed bioreactor**. It consists of a packed bed of inert material, on which the microorganisms adhere and form a biofilm. Water is continuously sprayed on the packed bed and trickles down in co-current or

counter-current flow with the air (Figure 2B). Due to this water phase the humidity, the temperature, and the supply of nutrients can be controlled better than in a biofilter and mineral end-products can be removed. Trickle-bed reactors have been tested successfully on a semi-industrial scale to treat waste gases containing a variety of pollutants, including chlorinated hydrocarbons, aromatic compounds, alcohols, aldehydes and ketones, ammonia and sulphur compounds (6).

Excessive biomass growth in the trickle-bed bioreactor can lead to clogging of the filterbed, an increase in pressure drop and a decrease in reactor performance. Recently, various control strategies have been tested to prevent clogging, as there are limitation of the nitrogen supply, the use of nitrate instead of ammonium, back-washing, washing with NaOH, and modification of the inoculum (22, 28).

A disadvantage of the trickle-bed reactor is the water phase trickling down the biofilm. This layer of water forms a barrier for the transfer of poorly water-soluble pollutants from air to the biomass and makes the trickle-bed bioreactor unsuitable for the efficient removal of poorly soluble volatiles from waste gases (27).

Similar to the trickle-bed reactor the **bioscrubber** consists of a bed of inert packing material, over which water is sprayed. But now the biomass is not exclusively present on the packing material. The water phase absorbs the contaminants from the gas phase and is then transported to a separate tank, where biodegradation takes place. As contaminants have to dissolve in water, this type of reactor is not suitable for compounds with a low water solubility (27). In order to improve the mass transfer of poorly water soluble pollutants the use of organic solvents as absorbents has been proposed (2, 21).

A fourth type of bioreactor studied is the **dry biobed**. It consists of a packed bed of activated carbon that is sprayed occasionally. This type of reactor can be considered to be a combination of a biofilter and a trickle-bed reactor. It has been successfully tested on laboratory scale for the removal of the poorly water soluble pollutants ethene (4) and styrene (3).

For the removal of poorly soluble and/or chlorinated hydrocarbons from air a new type of bioreactor was proposed: the **membrane bioreactor** (Figure 2C) (12). Such a bioreactor consists of a hydrophobic microporous membrane that separates the gas phase and the liquid phase. Biomass grows on the "liquid" side of the membrane and pollutants and oxygen diffuse through the membrane. In a membrane bioreactor the advantages of both the biofilter and the trickle-bed reactor are combined.

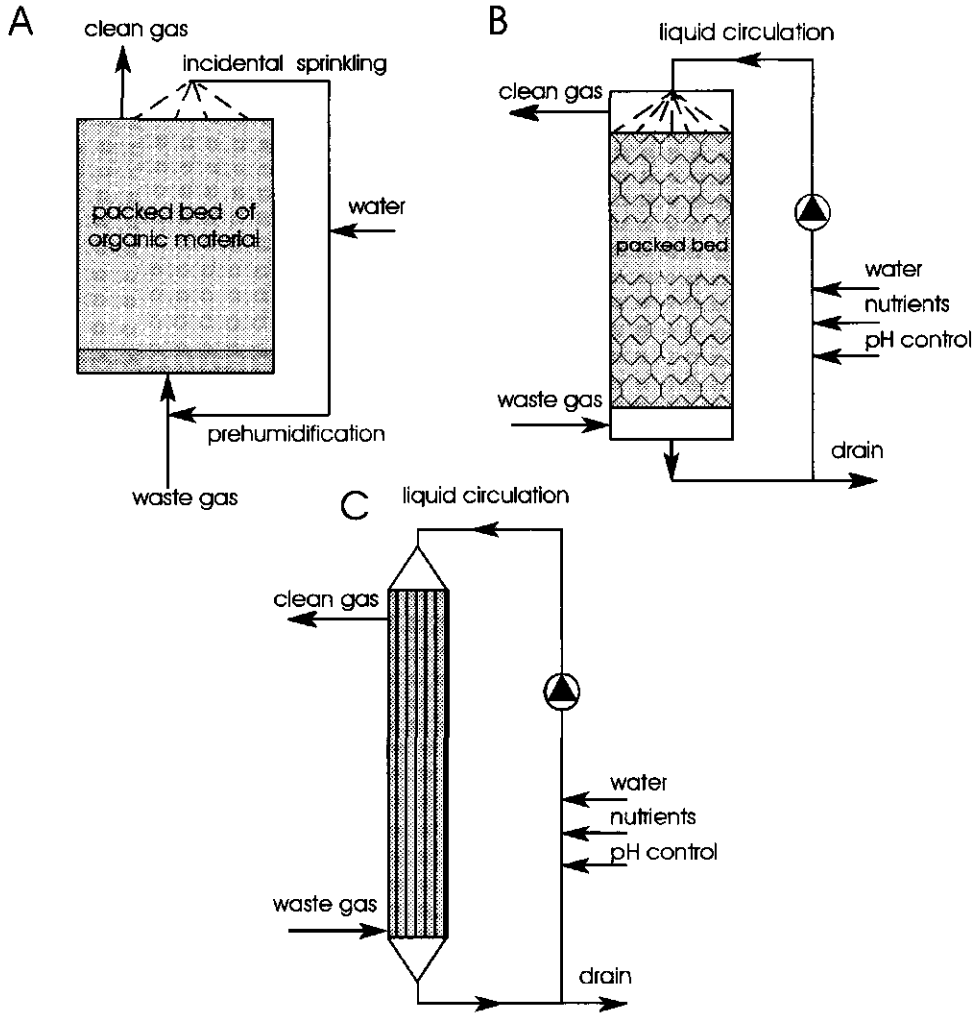

Figure 2. Schematic representations of various bioreactors for waste gas treatment. (A) biofilter, (B) trickle-bed reactor and (C) membrane bioreactor.

Just like the biofilter, the membrane bioreactor has excellent mass transfer properties, making it suitable for the removal of poorly soluble hydrocarbons. Similar to the trickle-bed reactor, a discrete liquid phase enables the removal of toxic end-products and the supply of nutrients and water. In Chapter 2 the membrane bioreactor for waste gas treatment, the use of membrane (bio)reactors in general, and several types of membrane material are described in detail.

Pollutants that are difficult to remove from air

Although biological treatment has been shown to be successful with a variety of pollutants, several classes of pollutants remain difficult to remove from air.

An important class of pollutants that are difficult to remove from air, are the **poorly water soluble volatiles**. A low water solubility results in low concentrations in the water phase, which surrounds the microorganisms. The degradation thus will be limited by a small driving force for mass transfer. Because of this limitation, the thickness of the water layer in between the gas phase and the microorganisms should be minimized. The design of the bioreactor in this respect is critical (19). The design of the membrane bioreactor will be discussed in detail in Chapters 2 and 3. A low water solubility also poses problems to the microbial population, especially if low concentrations of pollutant have to be removed from air. Microorganisms, that are present in the water phase, then experience a very low concentration of substrate and their activity is limited. This aspect is elaborated on in Chapter 5.

A second obstacle for biological waste gas treatment can be **lack of biodegradability**. Some chlorinated organic pollutants, for example, can not be degraded aerobically and consequently their removal from air (≈ 20% oxygen) is difficult. Other chlorinated hydrocarbons can be degraded under aerobic conditions, but only cometabolically. Their removal requires the addition of a second substrate for microbial growth (7).

The third aspect that can be problematic in biological waste gas treatment is **toxicity**. High concentrations of a pollutant occuring occasionally in the waste gas, can be toxic to the microbial population. High concentrations of intermediates formed by biological degradation can have adverse effects as well; Devinny and Hodge (5) described the accumulation of acidic intermediates in a biofilter that was overloaded with ethanol. Not only high concentrations are problematic. Low concentrations of pollutants may cause toxicity problems as well. Intermediates of aerobic trichloroethene biodegradation, for example, are known to have deleterious effects on the microorganisms degrading trichloroethene (10, 13). Moreover, mineral end-products accumulating in a bioreactor can gradually poison the biomass.

Concluding it can be said the removal of pollutants from air can be hampered by a poor water solubility, by lack of biodegradability and by toxicity. This thesis focusses mainly on the first aspect, while in Chapter 6 the other aspects are considered as well.

Pollutants studied in this work

The major part of this thesis deals with the removal of the poorly soluble hydrocarbon propene from air and in Chapter 6 the degradation of a second pollutant, trichloroethene, is discussed.

Propene is difficult to remove from air because of its poor water solubility; In 1 liter of water there is 10 times less propene than in 1 liter of air (at equilibrium at 30° C) (Chapter 3). Before propene can be degraded by microorganisms, it has to be transferred to the liquid phase and limitation of the reaction by mass transfer to the liquid is likely to occur. Therefore propene is a suitable model substrate to test the *mass transfer properties of a bioreactor for waste gas treatment.*

Propene (C_3H_6) is an unsaturated hydrocarbon and predominantly enters the atmosphere due to human activities, although biological production from soil has been reported (11). It is used for the production of fabricated polymers, fibres, and solvents and in the production of plastic products and resins. Its carcinogeneity is questionable (16). According to the Dutch emission standards propene has to be reduced to a concentration lower than 150 mg m^{-3} (90 ppm) if the total mass flow is more than 3 kg hr^{-1} (24).

Trichloroethene (TCE) is a suspected carcinogen (16) and has been widely used as solvent and degreasing agent. Although its use had decreased, the annual production and emission in 1992 was still 197,000 (± 10,000) metric tonnes (17). TCE is a widely distributed pollutant of groundwater and soil. Treatment of contaminated sites by air stripping or vapour extraction results in contaminated gas streams. Biodegradation may be an attractive alternative to physical-chemical treatment of these gas streams, such as adsorption on activated carbon (23). In the Netherlands TCE in off-gas has to be reduced to a concentration lower than 100 mg m^{-3} if the total mass flow is larger than 2 kg hr^{-1} (24).

Under aerobic conditions TCE can only be degraded cometabolically and its degradation results in inactivation of the biomass (7). Therefore, a constant supply of growth substrate is required to maintain the microbial population. If growth substrates and TCE are supplied simultaneously in the bioreactor, competition between the two substrates for the monooxygenase will occur and the TCE degradation rate can decrease dramatically (7). Moreover, the use of large amounts of volatile growth substrate required for biomass growth (see Chapter 6) might induce an additional source of air pollution. Therefore, the waste gas containing TCE and the growth substrate should be kept apart. This can be

achieved in specially designed membrane reactors in which the growth substrate is supplied in a closed loop. Several aspects of continuous TCE degradation and the potential of membrane bioreactors for removal of this recalcitrant compound from air, are discussed in the Chapters 2, 6 and 7.

Xanthobacter Py2

Both propene and TCE can be degraded by the same microorganism, the yellow bacterium *Xanthobacter* Py2, which was isolated with propene as the sole source of carbon and energy (26). The biodegradation of propene by *Xanthobacter* Py2 has been studied extensively by several groups focussing on the physiology (1, 29) and the genetics (25, 30) of the first two steps in the degradation pathway. The first step in the degradation of propene (Figure 3) is an oxidation to propene oxide and is performed by a propene monooxygenase (26). Eventually propene is oxidized to CO_2 and H_2O or incorporated into new biomass. Due to the aspecificity of the propene monooxygenase (Figure 3), *Xanthobacter* Py2 is also able to degrade trichloroethene co-metabolically (8).

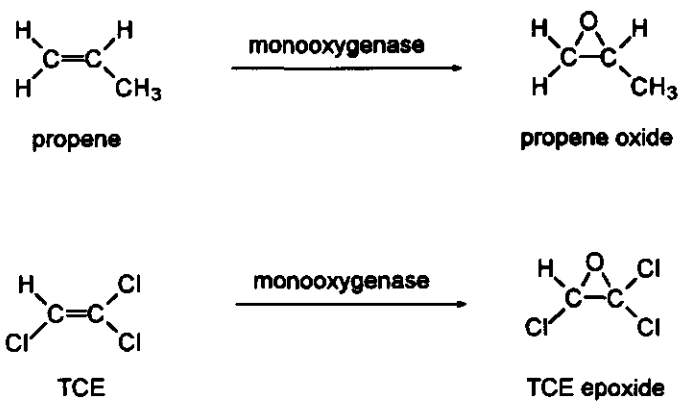

Figure 3. First step in the degradation of propene and trichloroethene by *Xanthobacter* Py2

Outline of this thesis

For the removal of poorly soluble pollutants from air, a bioreactor with a microporous hydrophobic membrane was designed and tested. Chapter 2 describes this type of reactor and gives an overview of literature concerning gas-

liquid processes in which membranes have been used. In Chapter 3 the mass transfer properties of the membrane bioreactor were tested with the poorly soluble model pollutant propene. Long-term stability of propene removal in hollow fibre membrane bioreactors and the effect of the nitrogen supply are discussed in Chapter 4. Chapter 5 deals with the kinetics of propene degradation, with growth at very low substrate concentrations and with the operation of a membrane bioreactor at concentrations required in off-gas treatment. Cometabolic degradation of trichloroethene and the problems associated with its toxicity, are described in Chapter 6. Finally, in Chapter 7 the potential applications of membrane bioreactors for the removal of recalcitrant pollutants are discussed.

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CHAPTER 2

Membrane bioreactors for waste gas treatment

Martine W. Reij, Jos T.F. Keurentjes, and Sybe Hartmans

Summary: This review describes the recent development of membrane reactors for biological treatment of waste gases. In such a bioreactor gaseous pollutants are transferred through a membrane to the liquid phase, where microorganisms degrade the pollutants. The membrane bioreactor combines the advantages of membrane devices with the clean technology of biological air purification.

Two types of membrane materials can be used for gas-liquid contact: hydrophobic microporous material and dense material, such as silicone rubber. Microporous material generally has a higher permeability, but dense membranes can be advantageous in the case specific selectivity is required.

Biomass is generally present as a biofilm on the membrane, but may also be suspended in the liquid phase. In a number of cases the reactor performance appears to be hampered by an unstable biofilm performance and/or by clogging of the liquid channels due to excessive biomass formation. So far, membrane bioreactors for biofiltration have only been tested on lab-scale. If the long-term stability of these reactors can be demonstrated, we expect membrane bioreactors to be useful tools in the treatment of gas streams containing poorly water-soluble pollutants and highly chlorinated hydrocarbons, which are difficult to treat with the conventional methods for biofiltration.

Introduction

The application of membranes has been proposed for a variety of purposes in waste management, as there are the separation of solids, biomass retention, aeration of bioreactors, and extraction of pollutants from wastewater. These processes were recently reviewed by Brindle and Stephenson (10). Here, however, we will focus exclusively on the removal of pollutants from air and the subsequent biodegradation in membrane reactors.

In bioreactors for waste gas treatment, organic pollutants diffuse into the liquid phase, where microorganisms degrade the pollutants into harmless products like CO_2, H_2O and minerals. Membrane bioreactors were designed as an alternative for conventional types of bioreactors for waste gas treatment, like the compost biofilter. An advantage of the membrane bioreactor over the biofilter is the presence of a discrete water phase allowing optimal humidification of the biomass and removal of degradation products, thus avoiding inactivation of the biomass.

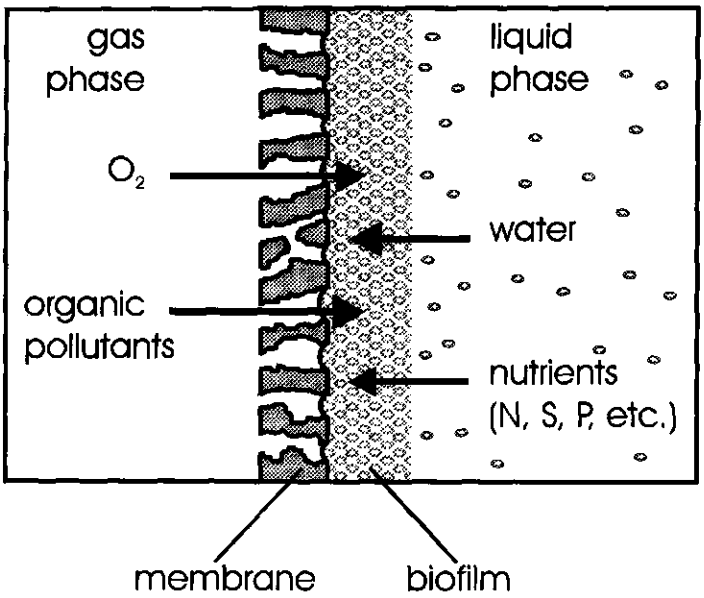

Figure 1. Schematic view of a membrane bioreactor for waste gas treatment containing a microporous hydrophobic membrane, a biofilm and suspended cells.

In a membrane bioreactor for waste gas treatment (Figure 1) the membrane serves as the interface between the gas phase and the liquid phase. The gas-liquid interface that can thus be created e.g. in hollow fibre reactors is larger than in other types of gas-liquid contactors (39). Moreover, membrane reactors do not contain moving parts, are easy to scale up, and the flows of gas and liquid can be varied independently without the problems of flooding, loading, or foaming commonly encountered in bubble columns (35).

In this review we describe a new type of gas-liquid contactor, in which the excellent mass transfer properties of membrane devices are combined with the clean technology of biological air purification. Based on a critical evaluation of these systems, the potential application niches will be defined.

Theory

Biological waste gas treatment can be described as an extraction of the gas phase with water, followed by consumption of the biodegradable components. The flux of a volatile component over the membrane in such a gas-liquid extractor is:

$$J = K_l \times A \times (C_g / m - C_l) \qquad \text{(Eq. 1)}$$

in which	J	= flux through the membrane	[mol s^{-1}]
	K_l	= overall mass transfer coefficient based on concentrations in the liquid phase	[m s^{-1}]
	A	= membrane surface area	[m^2]
	C_g, C_l	= concentrations in the gas and liquid phases	[mol m^{-3}]
	m	= air/water partition coefficient (concn. in gas [mol m^{-3}] / concn. in water [mol m^{-3}])	[-]

The concentration difference between the gas phase and the liquid phase provides the driving force for diffusive transport across the membrane. A pressure difference is not applied. The driving force depends strongly on the air-water partition coefficient (m) of the diffusing volatile. For components with a high partition coefficient (high m) the driving force for mass transfer is small. The concentration in the liquid (C_l), which depends on the biodegrading activity of the microbial population, also affects the driving force. The surface of the membrane (A) forms the contact area.

Two types of material have been used to prevent mixing of the gas and liquid phases and simultaneously allow the transfer of volatile components: hydrophobic microporous material and dense material. The properties of both types of membrane and the mechanisms involved, are discussed in the next two paragraphs.

Microporous membranes

Hydrophobic microporous membranes (Figure 2A) consist of a polymer matrix of e.g. polypropylene or teflon and contain pores with a diameter in the range of 0.01-1.0 μm. Since the membrane material is hydrophobic, the pores are filled with gas. Water does not enter the pores, unless a certain critical pressure at the liquid side is exceeded. For cylindrical pores this critical pressure (ΔP) is:

$$\Delta P = (2\gamma \cos\theta) / R \qquad \text{(Eq. 2)}$$

in which	γ	= interfacial tension between gas and liquid	[N m^{-1}]
	θ	= contact angle with polymer surface	[deg]
	R	= pore radius	[m]

The overall mass transfer resistance [$1/K_l$] for gas-liquid transfer is a combination of several resistances in series and for a gas-filled microporous membrane it is defined by:

$$\frac{1}{K_l} = \frac{1}{k_g \times m} + \frac{1}{k_m \times m} + \frac{1}{k_l} \qquad \text{(Eq. 3)}$$

in which	k_g	= mass transfer coefficient in gas phase	[m s^{-1}]
	k_m	= mass transfer coefficient in membrane	[m s^{-1}]
	k_l	= mass transfer coefficient in water phase	[m s^{-1}]

Both k_l and k_g depend on the flow rates in the individual phases but the liquid phase coefficient (k_l) also depends on the reaction rate. The mass transfer coefficient inside the membrane can be calculated as follows:

$$k_m = \frac{D \times \varepsilon}{\delta \times \tau} \qquad \text{(Eq. 4)}$$

in which	D	= diffusion coefficient	[m^2 s^{-1}]
	δ	= membrane thickness	[m]

ε = porosity of the membrane [-]

τ = tortuosity of the membrane [-]

The porosity is the fraction of pores in the membrane and the tortuosity is a measure for the shape of those pores. If the pores are large enough, interaction with the membrane material may be neglected and the diffusion coefficient in such a membrane (D) is equal to the diffusion coefficient in air (D_g). As soon as k_m/D_g has been determined for one or more volatiles, the resistance of that membrane for any volatile can be calculated using D_g. If interaction can not be neglected, an effective diffusion coefficient has to be calculated (23).

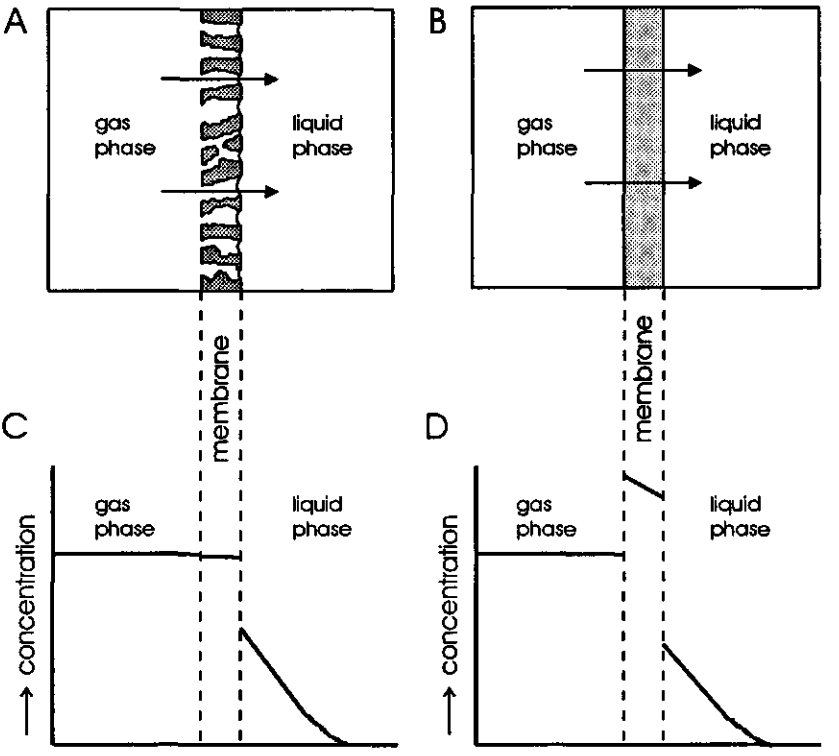

Figure 2. Membrane materials for gas-liquid contact: (A and C) hydrophobic microporous material and (B and D) dense material. C and D represent concentration profiles of a volatile diffusing from the gas phase across the membrane to the liquid phase, where it is consumed. Not on scale.

Dense membranes

In case of transport through a dense membrane, the diffusing volatile is absorbed in the membrane material and diffusion takes place in the dense polymer (Figure 2B). The overall mass transfer coefficient for gas-liquid transfer is:

$$\frac{1}{K_l} = \frac{1}{k_g \times m} + \frac{1}{k_m} + \frac{1}{k_l} \qquad \text{(Eq. 5)}$$

The mass transfer coefficient inside dense membrane (k_m) depends on both the solubility and the diffusivity of a volatile in the dense matrix (13), according to :

$$k_m = P / \delta = S_m \times D_m / \delta \qquad \text{(Eq. 6)}$$

in which	P	= permeability of dense membrane	[$m^2\ s^{-1}$]
	S_m	= solubility	[-]
	D_m	= diffusion coefficient in the membrane	[$m^2\ s^{-1}$]

The solubility is the concentration in the membrane (in mol m^{-3}) divided by the equilibrium concentration in the water phase (in mol m^{-3}). For each volatile the solubility and diffusivity are different and the mass transfer resistances of dense membranes for various gases may differ considerably due to specific interactions between the components in the gas phase and the membrane material. As a consequence, selected components can be selectively extracted from or retained in the gas phase by a proper choice of the membrane material.

Gas-liquid contactors

Both microporous and dense membranes have been used for a variety of processes that involve gas-liquid contact. Microporous material is generally applied in hollow fibres, although spiral-wound and plate-and-frame modules have also been used (35, 38). Microporous membranes can be applied as gas-liquid contactors when selectivity is not required and only provide gas-liquid contact area.

Dense material is available as tubes (usually silicone tubing), with a wall-thickness of at least several hundreds of micrometers, and in composite membranes. Composite membranes consist of a thin, selective toplayer (< 1 μm to 30 μm) of dense material,

supported by a highly porous support layer of e.g. non-woven polyester or a microfiltration membrane. Composite membranes can be applied in spiral-wound, plate-and-frame and in hollow-fibre modules (29).

Examples of the application of both microporous and dense membranes as gas-liquid contactors are given in the next two paragraphs. It should be noted that the list of examples is by no means complete.

Gas-liquid contactors with microporous membranes

Microporous membranes were first used in blood-oxygenation. Blood flows on one side of the membrane and air or pure oxygen flows on the other side. In this way oxygen is supplied to and carbon dioxide is removed from the blood during surgery (15, 35). Other applications of membrane reactors for oxygen transport are the cultivation of shear-sensitive cells (5, 37), fermentors with a high oxygen demand (6), and reactors for wastewater treatment (for a review see [10]). On the other hand, hollow-fibre contactors were also tested as artificial gills, by which dissolved oxygen could be removed from water and small animals could breathe under water (39,40).

Microporous fibres were also applied in gas-absorption. During (non-biological) gas-absorption the contaminants, such as sulphur dioxide or ammonia, are transported through the membrane, dissolve in the liquid phase and disappear quickly by chemical reaction (20, 28). Membrane reactors provide a larger surface area per volume than conventional gas-liquid contactors and consequently allow higher removal rates. A ten-fold increase in efficiency was observed when using a hollow-fibre device over conventional packed columns (39).

Gas-liquid contactors with dense membranes

Dense membranes have been tested primarily for aeration purposes. Of the various types of dense material, silicone rubber (polydimethylsiloxane, PDMS) was found to have the highest oxygen permeability (13) and this material was used for the aeration of wastewater (10).

For wastewater oxygenation dense hollow fibres were preferred over microporous material since oxygen can be supplied at elevated pressure, thus increasing the driving force for mass transfer (11). With microporous membrane the pressure of the gas phase can not be increased, because gas bubbles will appear in the liquid. For waste gas treatment, however, increasing the gas phase pressure is not relevant.

Other gas-phase components than oxygen can be transported through dense membranes as well. Pervaporation is widely used for the selective transfer of volatile components from a mixture to the gas phase. Depending on the properties of the component to be transported, a suitable polymer can be selected (7,36). Using selected polymers, solvents were extracted from aqueous solutions (9,25).

Finally, dense membranes were tested for gas absorption. Acetone was recovered selectively from air streams using a composite membrane consisting of a thin layer of PDMS in a polysulfone hollow-fibre carrier (22).

Applications in biological waste gas treatment

In addition to non-biological gas absorption, membrane contactors have recently been tested for biological treatment of gas streams. In such a process the pollutants diffuse through the membrane and are degraded by the microbial population present in the liquid phase (Figure 1). An overview of publications on this subject is shown in Table 1.

In general, the biomass is supplied with carbon and oxygen from the gas phase, while water and mineral nutrients are supplied by the liquid phase. Microorganisms grow as a biofilm on the membrane, but may also be suspended in the liquid phase. Figure 3 shows such a biofilm, grown on a microporous hydrophobic membrane.

Most studies mentioned in Table 1 concern the removal of hydrophobic pollutants from air. Hydrophobic pollutants, like xylene, toluene, hexane, and propene, have a high air-water partition coefficient (m). As can be seen in equation 1, the driving force for the transfer of these pollutants to the water phase is very small and as a consequence the biodegradation is limited by mass transfer and the design of the bioreactor is critical. The large gas-liquid interface and excellent mass transfer properties of membrane reactors (20, 39) have inspired several workers to test membrane bioreactors for the removal of poorly water soluble pollutants from air (4, 17, 32, 34).

The membrane materials used in several studies, were chosen such, that they were impermeable to microorganisms (16, 17). As a consequence, these organisms can not contaminate the gas phase. This precaution is considered to be important in case the membrane bioreactor is applied for the treatment of indoor air. For the same reason the membrane bioreactor was also selected for even more demanding conditions, such as in the manned space cabin (8).

Table 1. Membrane bioreactors for biological waste gas treatment in historical order.

Compound to be removed from air	Concn. (ppm)	Type of membrane	Nutrient supply in liquid	Biofilm	Inoculant	Reference number
Xylenes	30-140	silicone; tubes	minerals	yes	sludge	3
n-Butanol	40-180	*idem*	*idem*	yes	*idem*	*idem*
Dichloromethane	60-220	*idem*	*idem*	no	*idem*	*idem*
Toluene	20	HM [a]; sheet	minerals	NA [b]	*Pseudomonas* GJ40	17
Dichloromethane	47	*idem*	*idem*	NA	strain DM21	*idem*
Toluene	56	HM; sheet	minerals	yes	*Pseudomonas* GJ40	31
Dichloromethane	69	*idem*	*idem*	yes [c]	strain DM21	*idem*
n-Hexane	32	silicone; tubes [d]	minerals	?	?	34
Toluene	32	*idem*	*idem*	?	?	*idem*
NO	5	H; sheet	alcohols, minerals	yes	*Methylobacter*	19
Mixture	"low"	HM; sheet	minerals	yes	various strains	8
Dichloroethane	150	silicone; spiral wound	minerals	yes	*Xanthobacter* GJ10	16
Propene	250-3000	HM; sheet	minerals	yes	*Xanthobacter* Py2	32
Trichloroethene	20	polysulfone; fibers	acetate, minerals [e]	yes	sludge	27
Propene	330-2700	HM; fibres	minerals	yes	*Xanthobacter* Py2	33

[a] H = hydrophobic material; M = microporous material.
[b] Experiments lasted less than 1 day.
[c] Severe sloughing observed after 4 days.
[d] Reactor is a combination of a membrane bioreactor and a bubble-column and was designed for simultaneous degradation of both hydrophylic and hydrophobic contaminants from the gas phase.
[e] Liquid phase was kept anaerobic.

In general, the pollutants diffusing through the membrane serve as carbon sources for the microbial population. However, Hinz et al. (19) removed nitrogen monoxide from tunnel air and supplied the carbon source for the microbial population in the liquid phase.

Air containing chlorinated solvents was treated in membrane reactors as well. Freitas dos Santos et al. (16) tested a reactor with silicone tubes to remove 1,2-dichloroethane from air. For the destruction of trichloroethene (TCE) Parvatiyar et al. (27)designed a new concept of a membrane bioreactor in which both an aerobic and an anaerobic region were present. In the anaerobic zone TCE is partially dechlorinated and the products are supposedly degraded further in the aerobic zone of the biofilm (Fig. 4).

The membrane may also provide protection for the microbial population. During the removal of nitrogen monoxide, the microbial population was protected by the membrane from heavy metals present in the air contaminated by vehicle exhaust gases (19). In a second example, silicone membranes due to their selectivity for hydrophobic components, retained acid vapors (SO_2) that could hamper biodegradation of 1,2-dichloroethane (16).

Dense membranes may also serve as a buffer, in case the supply of pollutants is variable. It should be noted, however, that due to simple thermodynamics, the equilibrium concentration in the water phase will never change upon the insertion of any type of membrane between the gas phase and the water phase.

All studies mentioned in Table 1 concern laboratory scale experiments. No reports have been found so far on pilot-plant investigations or full-scale applications of membrane reactors in biological waste gas treatment.

Comparison with conventional bioreactors for waste gas treatment

Membrane bioreactors were designed as an alternative for conventional types of bioreactors for waste gas treatment, like the biofilter, the trickle-bed reactor and the bioscrubber.

A biofilter usually consists of a bed of compost through which waste gas is blown. Microorganisms present in the compost degrade the organic pollutants in the gas. To prevent dehydration of the biofilter, the waste gas has to be prehumidified. Treatment of gases containing chlorinated pollutants, sulphur compounds or ammonia, results in the accumulation of chloride, sulphate, or nitrate ions and causes acidification of the

biofilter. Acidification can to a certain extent be prevented by the addition of a buffering agent, e.g. lime, but large amounts of mineral end-products can not be neutralized in nor removed from a biofilter. An advantage of the membrane bioreactor over the compost biofilter is the presence of a discrete water phase. Due to this water phase toxic end-products can be removed from the reactor, and the humidity, the temperature, and the nutrient supply can be controlled closely and independently from the gas flow.

Two other types of bioreactors used for waste gas treatment are the trickle-bed bioreactor and the bioscrubber. Both reactors consist of a packed bed of inert material on which water is continuously sprayed. In the trickle-bed reactor micro-organisms are present on the packed bed, while in the bioscrubber biodegradation is mainly located in a separate tank. Just like in the membrane bioreactor the conditions in these bioreactors can be controlled and end-products can be removed by means of the discrete water phase. However, the location of the water film with respect to the biomass, differs. In the trickle-bed reactor and in the bioscrubber pollutants have to diffuse through the water phase, before they can be consumed by the microorganisms. For pollutants with a poor water-solubility (large m), such a layer of water causes a substantial additional resistance for mass transfer (14). In the membrane bioreactor, on the contrary, the liquid phase is situated at the opposite side of the biofilm and hardly forms a barrier for mass transfer of the poorly water soluble pollutants (Figure 1).

As mentioned before, large gas-liquid interfaces of 1000 to 10,000 $m^2\ m^{-3}$ can be created in hollow-fibre reactors (29), allowing high mass transfer rates. The pressure drop in the gas phase is much lower than observed in biofilters, where the pressure drop may become significant (26).

Disadvantages of membrane bioreactors are the high investment costs, particularly compared to biofilters, and possible clogging of the liquid channels due the formation of excess biomass (33). Compared to other types of bioreactors, the membrane may form an additional barrier for mass transfer (equations 3 and 5). However, as will be shown in the next section, the mass transfer resistance of the membrane is often negligible.

Membrane resistance

The resistance of both silicone membranes and microporous hydrophobic materials for various volatiles are shown in Table 2. For silicone membrane material the data were taken from various sources in literature and for microporous membrane the value of k_m/D_g was used as determined by Reij et al. (32) for microporous polypropylene with a porosity of 70%-75%. For both types of membrane a thickness of 100 µm was assumed.

The resistance depends largely on the gas-liquid partition coefficient (*m*) of the diffusing volatile: a poor water solubility (large *m*) implies a small relative resistance in the membrane. From this table it can be concluded that the resistance of microporous membrane is 10 to 150 times smaller than the resistance of silicone membrane of the same thickness.

It should be noted that the mass transfer coefficient in a membrane is reciprocal with its thickness. For microporous membrane a thickness of 100 µm, as calculated in Table 2, is quite relevant, because the thickness of microporous fibres and flat membrane is usually in the range of 30 to 100 µm (23, 38, 39). For dense material, however, 100 µm is not always relevant. Silicone tubes have a wall thickness of 150 µm up to 1 mm (3, 12, 34) and the mass transfer coefficient of such tubes will be considerably smaller than the values mentioned in Table 2, thus making the resistance to mass transfer even more important.

On the other hand, when a composite material is used, a very thin layer (< 1 µm to 30 µm) of dense material is sufficient and the resistance of the dense layer can be reduced significantly. However, when using composite material the mass transfer characteristics of the backing material and its location should be considered as well. The backing may attribute to the mass transfer resistance, especially when located at the liquid side of the membrane. Moreover, the backing may get fouled with biofilm material. Therefore, when using composite membranes, the backing should preferably be located at the gas-side.

To examine the importance of the membrane resistance, the membrane resistance should be compared to the resistance in the liquid phase. The liquid phase coefficients (k_l) in well-mixed bioreactors like bubble columns and stirred tanks, are in the range of 5×10^{-5} to 5×10^{-4} m s^{-1} (21). In the membrane bioreactor mixing is less intensive than in the bioreactors tested by Kawase et al. (21), but on the other hand (bio)chemical reaction in the liquid phase enhances the k_l significantly.

Table 2. Estimation of the mass transfer coefficients of hydrophobic microporous membrane (32) and dense silicone (PDMS) membrane, both with a thickness of 100 μm.

Volatile	Partition [a] coeff. air/water (m) at 25°C [-]	Diffusion [b] coeff. in air at 25°C [$m^2 s^{-1}$]	Permeability of PDMS at 25°C [$m^2 s^{-1}$]	Reference for permeability of PDMS	Mass transfer coefficient based on aqueous conc. [$m s^{-2}$]	
					microporous	silicone
Oxygen	32	2.02×10^{-5}	3.9×10^{-7}	12	5.5×10^{-1}	3.9×10^{-3}
idem			2.4×10^{-8} [c]	36		2.4×10^{-4}
Propane	28	1.14×10^{-5}	1.7×10^{-8} [c]	36	2.7×10^{-1}	1.7×10^{-4}
Methane	27	2.12×10^{-5}	3.1×10^{-8} [c]	36	4.9×10^{-1}	3.1×10^{-4}
Ethene	8.8	1.51×10^{-5}	2.3×10^{-8} [c]	36	1.1×10^{-1}	2.3×10^{-4}
Carbon dioxide	1.2	1.59×10^{-5}	4.1×10^{-9} [c]	36	1.6×10^{-2}	4.1×10^{-5}
Trichloroethylene	0.48	8.33×10^{-6}	5.4×10^{-9}	25	3.4×10^{-3}	5.4×10^{-5}
idem			2.8×10^{-8}	18		2.8×10^{-4}
Toluene	0.26	8.15×10^{-6}	8.2×10^{-9}	25	1.8×10^{-3}	8.2×10^{-5}
Chloroform	0.18	9.05×10^{-6}	7.6×10^{-9}	18	1.4×10^{-3}	7.6×10^{-5}
Dichloromethane	0.10	1.04×10^{-5}	5.6×10^{-9}	18	8.9×10^{-4}	5.6×10^{-5}
Dichloroethane	0.05	9.15×10^{-6}	3.8×10^{-9}	18	3.9×10^{-4}	3.8×10^{-5}
Ethylacetate	0.0056	8.80×10^{-6}	1.9×10^{-10}	24	4.2×10^{-5}	1.9×10^{-6}
Acetone	0.00161	1.07×10^{-5}	1.4×10^{-10}	7	1.5×10^{-5}	1.4×10^{-6}
Propanol	0.00028	1.07×10^{-5}	3.7×10^{-11}	7	2.6×10^{-6}	3.7×10^{-7}
Ethanol	0.00021	1.24×10^{-5}	1.1×10^{-11}	7	2.2×10^{-6}	1.1×10^{-7}
Methanol	0.00018	1.67×10^{-5}	4.9×10^{-12}	7	2.6×10^{-6}	4.9×10^{-8}

[a] Dimensionless air-water partition coefficient at 25°C [mol m^{-3} per mol m^{-3}] (1).
[b] Diffusion coefficient in air at 25°C, calculated according to the method of Fuller et al. (30).
[c] Permeability at 35°C.

From the comparison of the values in Table 2 with the liquid coefficients by Kawase et al. (21), it can be estimated that 100 µm dense silicone membrane may form a significant resistance for any volatile. The resistance of 100 µm microporous membrane seems only significant for volatiles with a high water solubility ($m < \approx 0.05$), or in case the volatile is consumed very rapidly in the liquid phase.

Literature on non-biological gas absorption corroborates this estimation. Yang and Cussler (39) studied the absorption of carbon dioxide ($m = 1.2$) and oxygen ($m = 32$) in water and concluded that the resistance of microporous hollow-fibres ($\delta = 30$ µm) for the transfer of both compounds could be neglected, unless the mass transfer in the liquid phase was accelerated by chemical reaction and/or by extremely fast liquid flow. The absorption of ammonia, which has a very good water solubility ($m = 0.0025$), was indeed limited by the mass transfer resistance in the membrane (28).

Figure 3. Photograph of a biofilm grown with methanol and toluene on microporous hydrophobic membrane. The arrow indicates the interface between the membrane *(right)* and the biofilm *(left)*. The sample was frozen in liquid propane, fractured at -130°C, etched at -80°C for 5 minutes, sputtered with 5 to 10 nm of platina and observed at -170°C in a Low Temperature Emission SEM (JEOL 6300 F) at 5 kV. Bar = 1 µm.

Microbial growth in membrane bioreactors

Irrespective of the membrane resistance, the driving force for mass transfer depends on the concentration to which the pollutant is reduced in the liquid phase. Therefore, the removal rate in a membrane bioreactor depends largely on the activity of the microbial population.

As can be seen in Table 1, in most studies biofilm formation was observed or was even essential (34). Both mixed cultures and pure cultures formed biofilms. The hydrophobic nature of both microporous and silicone membranes facilitates microbial adhesion. The microorganisms located close to the membrane are exposed to higher substrate concentrations than suspended cells, making it more likely that cell growth occurs close to the membrane.

Biofilm growth may cause serious problems if excess biomass can not be removed. Freitas dos Santos et al. (16)attributed the decreasing reactor performance and the increasing pressure drop over the liquid phase to extensive biofilm formation in the spiral-wound membrane module. Hinz et al. (19) observed slime formation on a membrane with a high oxygen permeability. The slime resulted in clogging of the reactor. This problem was not observed when membranes with a low oxygen permeability were used. Clogging of hollow fibres with a biofilm of propene-degrading *Xanthobacter* could be prevented by applying a very high liquid velocity, but still the reactor performance decreased over a period of 3 to 6 months. These results suggest that, even if clogging is prevented, biofilms are prone to aging (33).

No biofilm was observed during the degradation of dichloromethane (3). A biofilm growing with the same pollutant sheared of the membrane after ± 4 days, causing a drop in reactor performance (31). The reason for both observations might be that hydrochloric acid, produced during the degradation of dichloromethane, accumulates in the biofilm to toxic levels and destabilizes the biofilm.

Aziz et al. (2) purposely repressed biofilm formation in a membrane reactor for wastewater treatment by the addition of a sequestering agent. This membrane reactor was part of a two-stage bioreactor, in which methanotrophs were circulated. In the membrane reactor the methanotrophs cometabolically degraded trichloroethylene (TCE) and in a separate reactor growth substrate was supplied, since TCE itself does not support microbial growth. Such a two stage process may also be used for the removal of TCE from waste gas.

A completely new strategy for the removal of TCE from air was designed by Parvatiyar et al. (27). In their membrane bioreactor acetate is added to the liquid phase as carbon source and as electron donor to lower the oxygen tension in the biofilm. Under the anoxic conditions that are created in this way, TCE can be partially dechlorinated. Subsequently the products of the anaerobic dechlorination are degraded further in the aerobic zone of the biofilm (Figure 4). Their work, however, does not contain experimental evidence that both the oxic and the anoxic zone are present, but it is the first report on the continuous removal of TCE from air in the absence of volatile growth substrates.

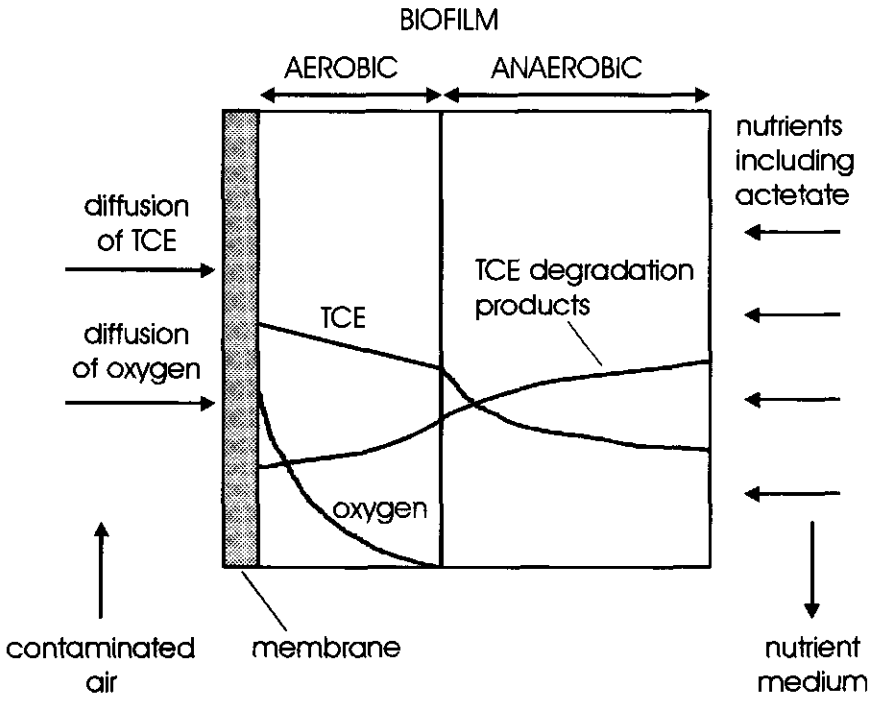

Figure 4. Combined aerobic and anaerobic degradation of TCE, showing the diffusion patterns of oxygen, TCE, and TCE degradation products. Reproduced from Parvatiyar et al. (27).

Outlook and conclusions

All studies presented in this review are lab-scale studies. By their modular nature, membrane modules are relatively easy to scale up (20), but before full-scale membrane modules can be applied in waste gas treatment, the long-term performance should be tested extensively.

The effects of biomass on the membrane material in the long run have not been tested sufficiently. During prolonged operation the pores of hydrophobic microporous membranes might be penetrated by biomass and/or water. To prevent penetration of the membrane pores, a thin coating of dense material could be applied on the liquid side of a porous membrane. Such composite membranes have been used in blood oxygenation to suppress blood-trauma and prevent the pores from filling with liquid and cell debris (35).

In addition to the durability of the membrane material, the stability of the biomass is essential as well. The formation of thick biofilms (16) and clogging of the liquid channels (19, 33) were shown to deteriorate the reactor performance. Even when clogging was prevented by a very fast liquid flow, the performance of hollow-fibre modules decreased in time (33). Therefore, strategies have to be developed to monitor the biofilm, to stabilize its activity, and to remove excess biomass from the membrane modules.

In the authors' opinion the removal of poorly water-soluble pollutants from air can be considered to be the most promising application for membrane bioreactors. The mass transfer resistance of membranes for this group of pollutants is negligible. Moreover, the large gas-liquid interface of membrane modules enables efficient removal of these pollutants, that in general are difficult to remove from air.

Other niches for the application of membrane bioreactors are indoor applications and the removal of pollutants that require a specific microbial population, like TCE and nitrogen monoxide. Very recently, a membrane bioreactor with both an anaerobic and an aerobic zone was proposed (27). Such a bioreactor might enable the biodegradation of pollutants, such as highly chlorinated hydrocarbons, that until now are considered to be beyond the reach of (aerobic) biological waste gas treatment.

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CHAPTER 3

Membrane bioreactor with porous hydrophobic membrane as gas-liquid contactor for waste gas treatment

Martine W. Reij, Kees D. de Gooijer, Jan A.M. de Bont, and Sybe Hartmans

Summary: A novel type of bioreactor for waste gas treatment has been designed. The reactor contains a microporous hydrophobic membrane to create a large interface between the waste gas and the aqueous phase. To test the new reactor propene was chosen because of its high air/water partition coefficient, which causes a low water concentration and hampers its removal from air. Propene transfer from air to a suspension of propene-utilizing *Xanthobacter* Py2 cells in the membrane bioreactor proved to be controlled by mass transfer in the liquid phase. The resistance of the membrane was negligible. Simulated propene transfer rates agreed well with the experimental data. A stable biofilm of *Xanthobacter* Py2 developed on the membrane during prolonged operation. The propene flux into the biofilm was 1×10^{-6} mol m^{-2} s^{-1} at a propene concentration of 9.3×10^{-2} mol m^{-3} in the gas phase.

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INTRODUCTION

Microporous hydrophobic membranes consist of a porous matrix in which gas is present. Water does not enter the pores unless a critical pressure is exceeded. Several applications of this type of membrane as gas-liquid contactor have been described and have recently been reviewed by Sirkar (22). Microporous hydrophobic membranes have been studied for the aeration of liquids e.g. for blood oxygenation (8), bubble-free aeration of shear-sensitive cell cultures (23) and fermentor aeration at high oxygen demand (1, 2, 5, 13). Yang and Cussler (29) used hollow fibre contactors for the de-aeration of water in an attempt to design artificial gills. A third application is the absorption of CO_2 and other volatiles in liquids (12, 15, 19, 20).

Here we describe a new application for microporous hydrophobic membranes: biological waste gas treatment. In Figure 1 a schematic view of the membrane bioreactor (MBR) for waste gas treatment is shown. Organic pollutants in air diffuse through the membrane into the water phase, where they can be degraded by microorganisms. Microorganisms can also grow on the membrane forming a biofilm. They are supplied with organic carbon and oxygen from the gas phase and with water and minerals from the liquid phase.

The MBR has several advantages for waste gas treatment: A large gas-liquid interface can be obtained in membrane reactors and the gas pressure drop over the reactor is much lower than observed in biofilters, commonly used for biological waste gas treatment (18). A second advantage over biofilters is the presence of a discrete water phase in the MBR allowing the removal of toxic degradation products. An example of a toxic product is hydrochloric acid, produced during the degradation of chlorinated hydrocarbons, which causes acidification in biofilters (18).

Other types of membrane bioreactors have been proposed for the removal of hydrophobic contaminants from waste gas (3) and wastewater (6, 16, 17). In these cases, dense silicon membranes were used. Dense membranes have a higher mass transfer resistance than microporous hydrophobic membranes (1) because the contaminants have to dissolve in the membrane material and diffuse through this material, while in the porous membranes diffusion is in air. Therefore, we have chosen microporous hydrophobic membrane material for our bioreactor for waste gas treatment and in a preliminary study we have selected a polypropylene membrane material (11).

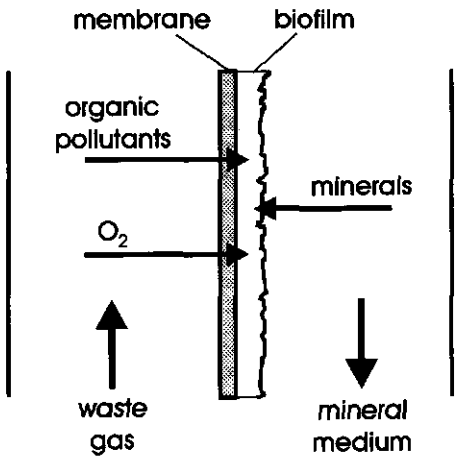

Figure 1. Principle of the membrane bioreactor for waste gas treatment.

In this study the performance of the membrane reactor was studied with propene and *Xanthobacter* Py2, which can grow aerobically with propene as sole source of carbon and energy, as a model system. Propene was chosen for its high air/water partition coefficient. Volatiles with a high partition coefficient are hard to remove from air by biological gas treatment, because of the low driving force for gas-liquid mass transfer.

MATERIALS AND METHODS

Organism and growth medium

Xanthobacter Py2 was previously isolated with propene as sole source of carbon (24) and was maintained on agar slants containing 3.5 g l^{-1} yeast extract and 5 g l^{-1} glucose. To obtain suspensions of cells, *X.* Py2 was grown at a dilution rate of 0.04 hr^{-1} in continuous culture with mineral salts medium (10) at pH 7.0 and 30°C with 2 % (v/v) propene in air as the carbon source, supplied at a rate of 0.1 vvm. Cells were harvested by centrifugation, washed once with 50 mM potassium phosphate buffer (pH 7.0) and suspended in the same buffer. Suspensions were used immediately.

Membrane material and chemicals

Propene (99.99%) and a gas mixture containing 0.4 % (v/v) propene and 0.5 % (v/v) CO_2 in air were purchased from Hoek Loos (Schiedam, The Netherlands). All other chemicals were reagent grade. The membrane material used in all experiments was hydrophobic polypropylene Accurel membrane, type 1E-PP, provided by Enka AG (Wuppertal, FRG). The porosity was 70-75 %, average pore diameter 0.1 µm and thickness 75-110 µm, as stated by the supplier.

Experimental set-up

The membranes were clamped between two identical halves of a perspex reactor as shown in Figure 2. The membrane separated the reactor into two compartments. Through one compartment liquid was pumped, while through the other compartment gas was blown. The effective membrane area was 40 cm^2 and the volume of both compartments was 8 cm^3. Gas flows were controlled by thermal mass flow controllers (Brooks Instrument, Veenendaal, The Netherlands). The set-up in Figure 3 was built to measure the overall mass transfer of propene from air to water. De-aerated water was pumped along the membrane (10b in Figure 3) at various velocities. Gas was circulated over the membrane at 1 l min^{-1} and the inflow of mixed gas was varied (0.15 to 3 ml min^{-1}). The gas loop was sampled at least three times during a period of 2 hours to confirm steady-state conditions and tested for leakage after each experiment. The whole set-up was placed in an isothermal chamber at 30°C, except for the condenser and the gas pump (KNF Neuberger, Freiburg, FRG).

For transfer with microbial reaction the de-aerated water was replaced by propene-grown suspended cells of *X.* Py2. The cell suspension was circulated (10a in Figure 3) over the membrane at a velocity of 4.1×10^{-2} m s^{-1}. Typical experiments lasted 3 hours and no nutrients were added.

With the same set-up a biofilm of *X.* Py2 was grown on the membrane inside the reactor. First the membrane reactor was sterilized with ethylene oxide. After removing residual ethylene oxide the reactor was inoculated for 2 days by circulating a propene-grown culture of *X.* Py2 over the membrane at 1.3×10^{-2} m s^{-1} in the set-up shown in Figure 3. After inoculation the pump was switched to the once-through mode and fresh mineral salts medium pH 7.0 (10) was pumped through the reactor continuously at a velocity of 2.1×10^{-4} m s^{-1}. *X.* Py2 grew on the membrane forming a biofilm.

Analytical methods

The propene concentrations in air were determined by analyzing headspace samples of 100 µl on a gas chromatograph equipped with a Porapak R column (100 - 120 mesh) at 210°C and a flame-ionization detector. All values are the average of at least three separate determinations. The standard deviation of the propene determination was less than 2 %.

The partition coefficient of propene over air and 50 mM potassium phosphate buffer at 30°C was determined in a manner similar to the EPICS method described by Gosset (9).

Maximum propene oxidation activities of suspensions of *X.* Py2 were determined in 130 ml serum flasks. Suspensions were diluted and 4 ml of the diluted suspensions were incubated with 1.0 % (v/v) propene in air at 30°C under vigorous shaking. Propene consumption rates of the suspensions were measured at two dilutions, the second dilution having a cell density twice as high as the first. The specific propene consumption rates (in nmol min^{-1} mg^{-1} dry weight) were found to be equal for both dilutions, confirming that under these conditions diffusion limitation did not occur.

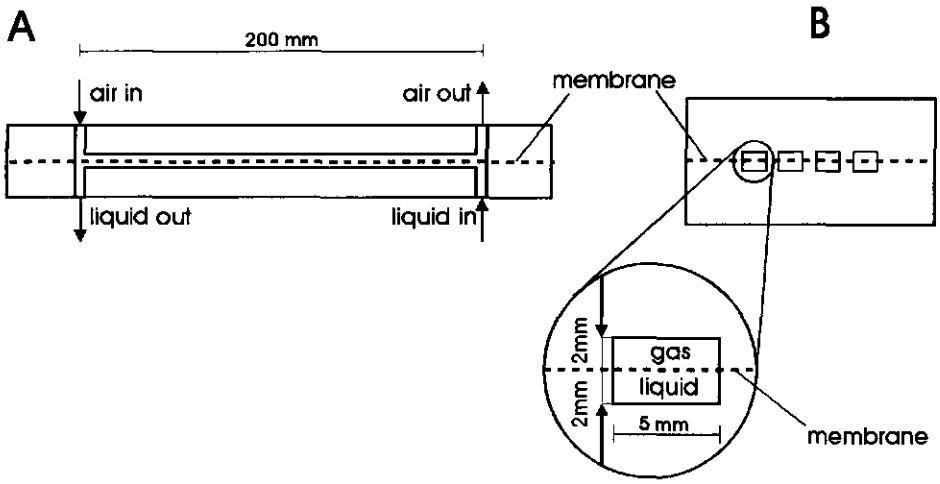

Figure 2. Membrane reactor (effective membrane area: 40 cm^2) with hydrophobic porous membrane clamped between two perspex halves. (A) longitudinal section, and (B) cross section.

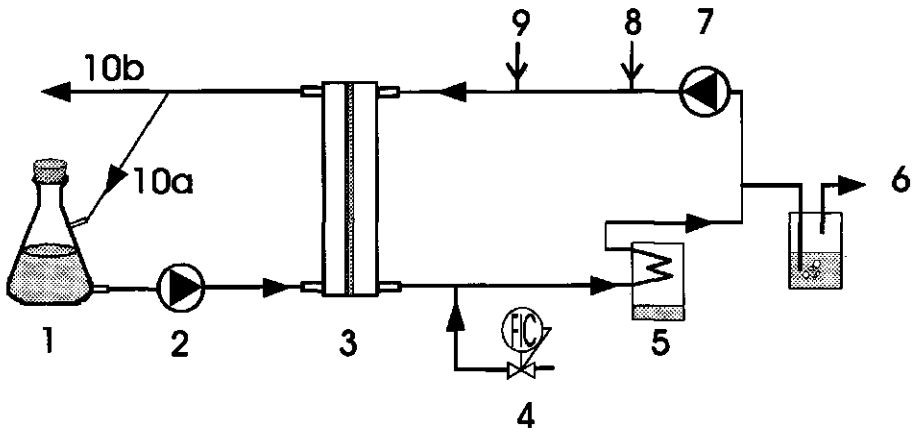

Figure 3. Experimental set-up for steady-state measurements of propene transfer from gas to liquid. 1: liquid reservoir, 2: liquid pump, 3: 40 cm^2 membrane reactor, 4: inflow of gas mixture with thermal mass flow controller, 5: condenser, 6: air overflow, 7: air pump, 8: sample port, 9: thermometer, 10: liquid (10a) circulation or (10b) outflow.

RESULTS AND DISCUSSION

Propene transfer to liquid in the absence of microbial reaction

Propene was chosen as a model component to test the performance of a lab-scale membrane reactor for the removal of a contaminant with a high air/water partition coefficient. The partition coefficient of propene (*m*) over air and water was found to be 10 ± 0.5 at 30°C.

First mass transfer of propene without reaction in the 40 cm^2 membrane module was investigated at various water velocities. In the absence of chemical reaction, the mass balance over the gas loop in Figure 3 at steady state is:

$$0 = \text{in} - \text{out} - \text{flux through membrane (J)}$$

$$0 = \phi \times C_{in} - \phi \times C_g - F \times C_{L\,outlet} \qquad \text{(Eq. 1)}$$

$$0 = \phi \times C_{in} - \phi \times C_g - k_{ov} \times A \times (C_g/m - C_l)^{log} \qquad \text{(Eq. 2)}$$

with

$$(C_g/m - C_l)^{log} = \frac{(C_g/m - C_{L,inlet}) - (C_g/m - C_{L,outlet})}{\ln(C_g/m - C_{L,inlet}) - \ln(C_g/m - C_{L,outlet})} \qquad \text{(Eq. 3)}$$

ϕ is the flow of mixed gas into the gas loop (no. 9 in Figure 3) and C_{in} is the propene concentration in the mixed gas. Both of these values and F, the flow of water through the liquid compartment of the reactor, are known. The concentration in the gas loop, C_g, was measured and the flux through the membrane (J) and the concentration of propene in the outflowing water ($C_{L,outlet}$) were calculated with equation 1.

The flux through the membrane is equal to $k_{ov} \times A \times$ driving force. The gas phase concentration (C_g) is determined experimentally and is constant over the length of the reactor since the gas phase is circulated at a flow rate exceeding ϕ more than 100 times. For the water phase plug flow without axial dispersion is assumed, because of the high Péclet number (10^6). Therefore the driving force can be described with the logarithmic mean $(C_g/m - C_l)^{log}$ to describe the concentration change in the water phase. The overall mass transfer coefficient was calculated with equation 2.

As can be seen in Figure 4 the dimensionless overall mass transfer coefficient (Sherwood number) depends on the liquid velocity according to:

$$Sh = 19 \times Re^{0.3} \qquad \text{(Eq. 4)}$$

In contrast with other gas-liquid contactors, as there are bubble-columns and trickle-bed reactors, a membrane is present between gas and liquid phases in the bioreactor under study (Figure 1). The overall mass transfer coefficient in this type of reactor is a function of the mass transfer coefficients in the gas, the membrane, and the liquid (22):

$$\frac{1}{k_{ov}} = \frac{1}{k_g \times m} + \frac{1}{k_m \times m} + \frac{1}{k_l} \qquad \text{(Eq. 5)}$$

For any volatile the membrane mass transfer coefficient can be calculated from D_g as described in Appendix 1 with $k_m/D_g = 9 \times 10^2\ m^{-1}$. For propene $k_m \times m$ is approximately $9 \times 10^{-2}\ m\ s^{-1}$. This means that the membrane mass transfer coefficient for propene is three orders of magnitude larger than k_{ov}. Therefore the resistance may assumed to be totally in the liquid phase resulting in k_{ov} being equal to k_l.

In the literature very few theoretical or experimental relations for flow along a flat membrane have been reported. In their review of mass transfer correlations Wicksramasinghe et al. (27) reported k_l to be proportional to $v^{1/3}$ for flow along a crimped flat membrane. This is quite close to our findings.

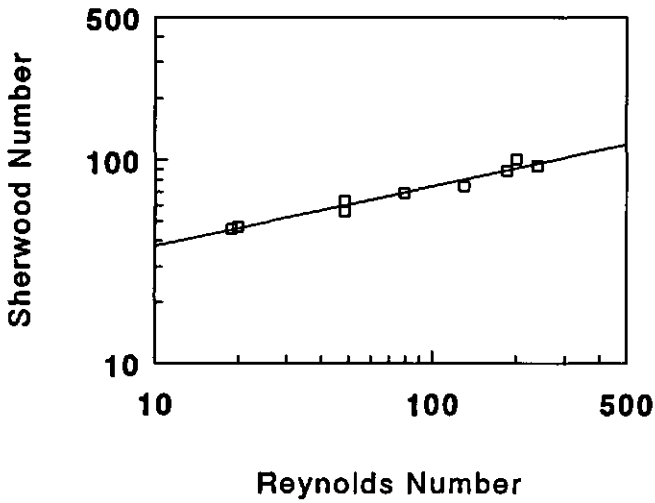

Figure 4. Sherwood number as a function of Reynolds number for transfer of propene to water in a flat sheet 40 cm^2 membrane reactor. Dimensionless groups as defined as follows: $Sh = k\ d\ /\ D_l$ and $Re = \rho\ v\ d\ /\ \mu$ with d = characteristic length equal to four times the cross section divided by the wetted perimeter.

Mass transfer of propene with microbial reaction

To determine the propene removal capacity of the 16 cm^3 membrane bioreactor, suspensions of the propene-consuming *Xanthobacter* Py2 were circulated through the reactor in the set-up shown in Figure 3. The maximum volumetric activity was varied by varying the cell density (1.6 to 11 g dry weight per liter). The maximum activity of the suspension was determined separately for every suspension in the absence of diffusion limitation as described in the Materials and Methods section. The concentration of propene in the gas loop was 9.6×10^{-2} (± 9%) mol m^3 during all experiments.

As can be seen in Figure 5 the flux of propene through the membrane increases with increasing cell density although not proportionally. However, the activities observed in the reactor were less than 1 % of the theoretical maximum consumption capacities of the biomass present in the reactor, demonstrating that mass transfer was limited by diffusion.

This is consistent with the findings of Qi and Cussler (20), who concluded from their experiments with O_2 and even CO_2, which is readily soluble in water, that the performance of microporous hollow fibre modules is nearly always controlled by mass transfer in the liquid phase, except when mass transfer is accelerated by chemical reaction or by very fast liquid flow.

Modelling propene transfer into a flowing suspension of cells

The liquid in the reactor can be described as liquid flowing over the membrane in the laminar flow regime. Propene diffuses through the membrane into the liquid and is consumed by the biomass according to Michaelis-Menten kinetics. Unfortunately no method is known to exactly calculate mass transfer rates as affected by both Michaelis-Menten kinetics and diffusion in laminar flow. Therefore we have calculated the upper and lower limits for comparison with the experimental data, as described in the next two sections.

Modelling transfer into flowing cells: Calculation of the lower limits.

The lower limits were found by calculating simultaneous reaction and diffusion in immobilized biomass with the simulation program BIOSIM (7). In this way the biomass is assumed to be immobilized, while it is actually flowing along the membrane. By neglecting the flow and mixing due to flow, this calculation underestimates the mass transfer rates and therefore is an estimation of the lower limits.

The principles of the BIOSIM program and the assumptions made in modelling this system are given in Appendix 2. Table 1 lists the input parameters used in the

simulations. The gas phase concentration (C_g) in the reactor and the maximal volumetric consumption rate (V_{max}) in mol per m^3 of liquid per second are determined separately for each suspension as described in the Materials and Methods section. For the diffusion coefficient (D_l) the free liquid value for water is assumed, not corrected for the presence of cells. The "biolayer thickness" is determined by the dimensions of the liquid compartment of the reactor (2 mm), which is filled completely with the cell suspension.

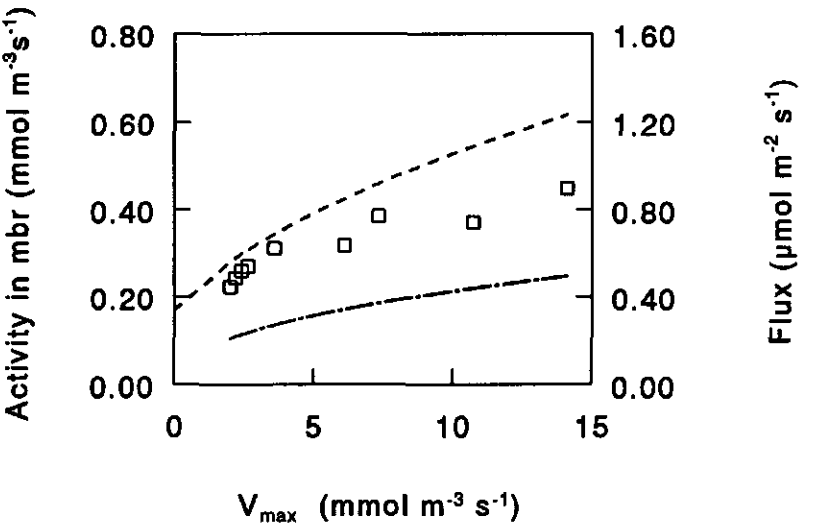

Figure 5. Consumption rates of propene by washed *X.* Py2 cells in the membranebioreactor in mmol per second per m^3 liquid phase in the reactor versus the maximum consumption rate of these cells. (□) experimental, (-•-•-) lower limits simulated with BIOSIM, assuming stagnant biomass, and (- - -) upper limits calculated with Hatta theory, assuming pseudo-first-order reaction.

Modelling transfer into flowing cells: Calculation of the upper limits

The upper limits in Figure 5 were found by using the Hatta theory and assuming the microbial degradation of propene to be a first order process. The Hatta theory describes the effect of chemical reaction on the mass transfer rate. From equation 4 the mass transfer coefficient in this MBR in the absence of reaction is known. Microbial reaction enhances the mass transfer rate by lowering the concentration in the liquid phase.

Assuming the degradation of propene by *Xanthobacter* Py2 to be a pseudo-first-order process, the first-order Hatta number, Ha, and the enhancement factor, E, in the flowing liquid can be calculated (4):

$$Ha = (D_l \times k_r / k_l^2)^{1/2} \quad \text{(Eq. 6)}$$

$$E = (Ha^2 + 1)^{1/2} \quad \text{(Eq. 7)}$$

The pseudo-first-order reaction constant k_r in equation 6 is defined as V_{max} / K_m and k_l is the mass transfer coefficient without reaction.

Assuming $k_l = k_{ov}$, k_l was calculated with equation 4 to be 3.9×10^{-5} m s^{-1} at the given liquid velocity of 4.1×10^{-2} m s^{-1}. Hence the flux into the liquid in the absence of biomass was calculated to be 0.17 mmol m^{-3} s^{-1}. In the presence of biomass the flux is enhanced by the factor E as given by equation 7.

Table 1. Input parameters for BIOSIM simulations.

Parameters	Definition	Value	Unit
V_{max}	max. volumetric cons. rate		mol $m^{-3}_{liq.}$ s^{-1}
	- in cell suspension (Fig. 5)	2×10^{-3} - 1.4×10^{-2} [a]	
	- in biofilm :	varied in Fig. 7	
K_m	Michaelis-Menten constant	1.09×10^{-3} (24)	mol $m^{-3}_{liq.}$
D_l	diffusion coefficient		m^2 s^{-1}
	- in cell suspension (Fig. 5)	1.4×10^{-9} (21)	
	- in biofilm	9.4×10^{-10} - 1.1×10^{-9} [b]	
C_g	gas phase concentration	0.01 - 0.12 [a]	mol m^{-3}_{gas}
R	thickness of biolayer	2.0×10^{-3}	m
m	partition coefficient	10	-

[a] Determined separately in each expermiment as described in Materials and Methods.

[b] Calculated according to the method recommended by Westrin (26), with specific activity 75 nmol min^{-1} mg^{-1} dry weight and cell volume 0.005 ml mg^{-1} dry weight.

Comparison of experimental and simulated data

In Figure 5 it can be seen that the experimentally observed transfer rates with flowing cell suspensions are about twice as high as the lower limits. Apparently some mixing occurs in the laminar flowing liquid. Sedimentation of cells, resulting in a higher biomass density close to the membrane (and hence a higher consumption rate) was ruled out as experiments with the reactor upside down gave the same transfer rates.

From Figure 5 it can be seen that the experimental values are indeed lower than the simulated upper limits. This was expected because in the Hatta calculations a pseudo-first-order reaction constant k_r was used, while the reaction obeys Michaelis-Menten kinetics (24) and the substrate concentrations in the liquid phase ($\pm 9.6 \times 10^{-3}$ mol m^{-3}) are essentially higher than the half saturation constant of the propene consumption (1.09×10^{-3} mol m^{-3}) (24). Therefore the reaction rate is overestimated with the help of the first-order Hatta numbers and this method yields the upper limits for the reaction rate.

Biofilm formation and activity

When the membrane reactor was operated overnight with a suspension of cells, formation of a thin yellow biofilm on the membrane was observed. Subsequently experiments were performed to investigate biofilm formation and the capacity of the membrane bioreactor for the removal of propene over a period of several weeks. In the first experiment the supply of gas mixture with 0.4% (= 0.16 mol m^{-3}) propene into the gas loop (no. 9 in Figure 3) was kept constant. After 5 days a biofilm of *Xanthobacter* Py2 had formed on the membrane and the concentration of propene in the gas loop had dropped to 9.3×10^{-2} ($\pm$ 9%) mol m^{-3}. In Figure 6 it can be seen that the flux of propene through the membrane into the biofilm was more or less constant during 20 days.

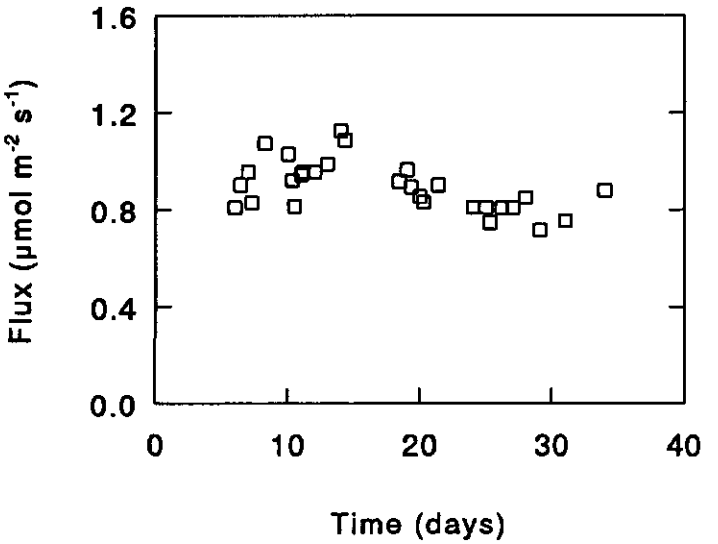

Figure 6. Flux into a growing biofilm of *X.* Py2 at constant propene concentration in the gas phase of 9.3×10^{-2} mol m^{-3}. The flux is expressed as µmol per second per m^2 membrane.

In a second experiment the rate at which the gas mixture with 0.4% propene was fed into the gas loop was varied, resulting in varying concentrations in the gas loop. In Figure 7 the propene flux into the biofilm is shown as a function of the propene concentration in the gas phase.

Comparison of the Figures 5 and 7 shows that the flux into the biofilm at 9.3×10^{-2} mol m^{-3} is about the same as the flux into suspensions of cells at the highest cell density tested (Figure 5). Since enhancement of mass transfer due to mixing is not possible in a biofilm, the biomass density of the biofilm has to be higher than the highest cell density studied with suspended cells, which was 11 kg m^{-3}.

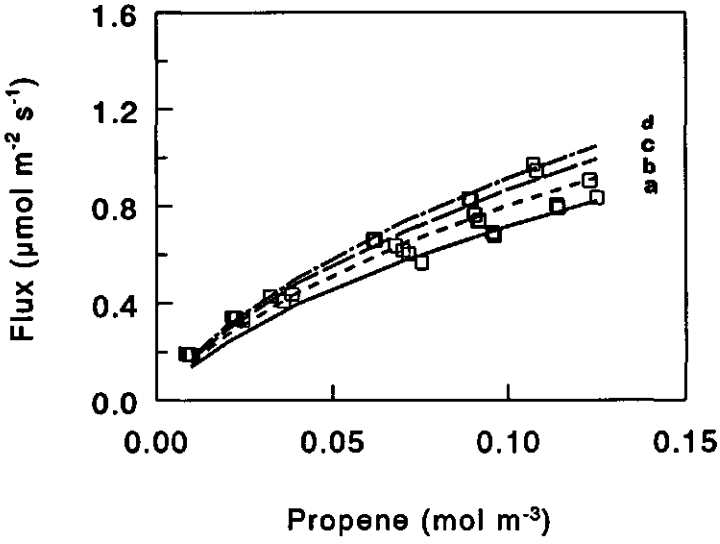

Figure 7. Flux into a biofilm of *Xanthobacter* Py2 as a function of the propene concentration in the gas phase. The flux is expressed as µmol per second per m^2 membrane. The lines a, b, c, and d represent simulated fluxes assuming maximal volumetric activities of 0.03, 0.04, 0.05, and 0.06 mol m^{-3} s^{-1} respectively.

Modelling propene consumption of biofilm

The value of the biomass concentration, expressed as maximum volumetric activity, (V_{max}) used in the simulation program BIOSIM was varied to find the best agreement with the consumption rates observed in the experiments at various propene

concentrations. The diffusion coefficient of propene in the biofilm was calculated according to the method recommended by Westrin (26). The effective diffusion coefficient was 67 to 78 % of the free liquid value depending on the biomass concentration (V_{max}) assumed. As can be seen in Figure 7 values for V_{max} of 4×10^{-2} to 6×10^{-2} mol m^{-3} s^{-1} gave the best agreement with the experimental data. Assuming the same specific activity as was determined for suspended cells, these consumption rates correspond to a biomass density of 32 to 48 kg dry weight per m^3 of biofilm. These values are well within the range of literature values. The actual biomass density is probably higher because cells in the biofilm probably have a lower specific activity than suspended cells freshly harvested from a continuous culture.

Variation of selected parameters in simulations

To test the impact of the parameters used in the BIOSIM simulation additional simulations were performed at the lowest (0.01 mol m^{-3}) and the highest (0.12 mol m^{-3}) propene concentrations shown in Figure 7. Throughout the simulation in the upcoming section a value for V_{max} of 4×10^{-2} mol m^{-3} s^{-1} was chosen, based on an optical fit of the data shown in Figure 7. Other input parameters are listed in Table I.

The external mass transfer coefficient for propene, describing the mass transfer resistance of the membrane, was calculated from appendix 1 to be 9×10^{-2} m s^{-1} and was used in the simulations shown in Figure 7. Neglecting the external resistance proved to have no effect on the simulated propene consumption rates. For compounds with a better water solubility (lower m), however, the value of k_m may form a significant part of the overall resistance, as can be seen in equation 5. For these compounds the membrane resistance may be in the same range as the resistance in liquid phase. For the poorly soluble compounds, like propene, the membrane resistance is negligible compared to the resistance in the liquid phase.

The thickness of the biolayer in Figure 7 was assumed to be 2×10^{-3} m, equal to the depth of the reactor module. The actual biofilm, however, was less than 2 mm thick. Therefore additional simulations were performed at decreased biolayer thicknesses (data not shown). At a concentration of 0.12 mol m^{-3} propene in the gas phase, decreasing the simulated biolayer thickness from 2000 µm to 50 µm resulted in less than 5% decrease of the propene transfer into the biofilm. At a concentration of 0.01 mol m^{-3} decreasing the thickness to 25 µm had no effect. This indicates that maximally 50 µm of this biofilm is actively involved in propene degradation. This value seems quite realistic for an aerobic biofilm, growing at a low substrate concentration.

For several parameters sensitivity analyses were performed by varying them from 50% to 150% percent of their original value. The impact of the variation in parameters is shown in Figure 8 for both a low (A) and a high (B) propene concentration.

Variation of Michaelis-Menten constant, K_m, hardly affects the consumption rate at high concentration (B). This is because the concentration of propene is considerably higher than the K_m. At the lower substrate concentration, which is very close to the K_m value, the effect of a change in the K_m is indeed larger.

Variations in the aqueous diffusion coefficient, D_l, and the maximum volumetric consumption rate, V_{max}, affect the consumption rate of the biofilm in a similar way. The effect of these parameters is equal for both the lower and higher substrate concentrations.

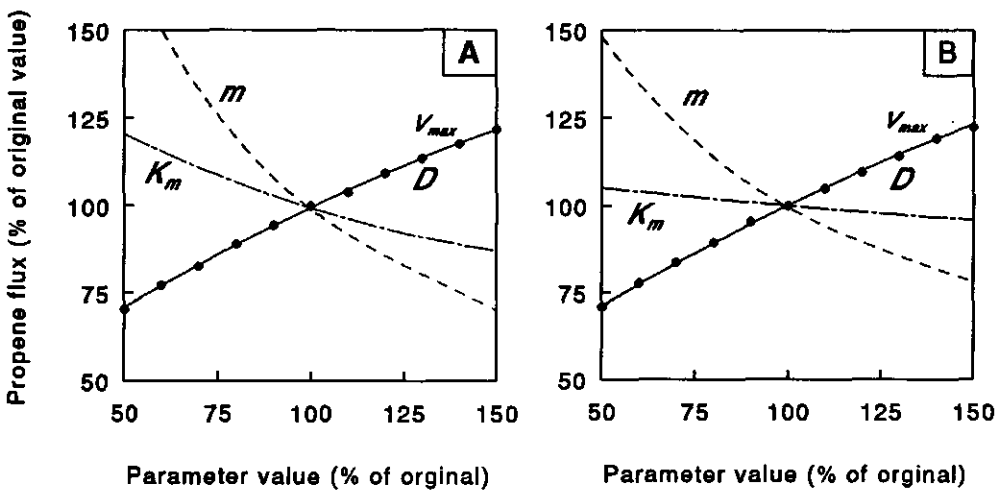

Figure 8. Sensitivity analysis: The effect of varying parameters on the calculated flux into the biofilm, expressed as µmol per second per m^2 membrane. Parameters varied are diffusion coefficient (D_l) (●--●), Michaelis-Menten constant (K_m) (-·-·-·-), partition coefficient (m) (- - - -), and maximum volumetric consumption rate (V_{max}) (———). Propene concentrations in the gas phase were set at (A) 0.01 mol m^{-3} and (B) 0.12 mol m^{-3} respectively.

A decrease in the partition coefficient, *m*, results in a considerable increase in the consumption rate. This can be explained by the direct effect the partition coefficient has on the substrate concentration in the liquid phase, since $C_l = C_g/m$. Therefore, *m* affects both the diffusion and the reaction rate. Figure 8 also illustrates that for substrates with a lower air/water partition coefficient a higher consumption rate can be expected in a biofilm at the same gas phase concentration. This observation has great impact on the practice of waste gas treatment. The efficiency of removal strongly depends on the aqueous concentration of the volatile to be degraded. Therefore besides the gaseous concentration, the air/water partition coefficient is an important factor determining removal rates in a gas-liquid reactor. The fact that pollutants with a high air/water partition coefficient are hard to remove from air has indeed been observed in waste gas treatment (18).

CONCLUSIONS

For the removal of propene with a membrane bioreactor the mass transfer resistance is totally in the liquid phase, the membrane resistance being negligible.

A stable biofilm of propene-degrading *Xanthobacter* Py2 formed on the membrane in the liquid compartment of the membrane reactor. The propene consumption by the biofilm was more or less constant for more than 20 days suggesting that practical application of this type of reactor for waste gas treatment is feasible, especially for the removal of volatiles with a high air/water partition coefficient.

The relationship between the propene concentration in the gas phase and the propene consumption rate of the biofilm could be described very well with the simulation program BIOSIM. With BIOSIM mass transfer in immobilized biomass as affected by both diffusion and reaction according to Michaelis-Menten kinetics is calculated.

Besides its potential applications in waste gas treatment, the membrane bioreactor can also be used for biofilm studies. It offers a unique tool for biofilm cultivation because nutrients can be supplied from both sides of the biofilm (Figure 1). Moreover the membrane bioreactor can be used to study microbial growth with poorly soluble volatile substrates, e.g. oxygen, without the problems of mass transfer limitation in the liquid phase usually encountered with biofilms growing on an inert surface.

ACKNOWLEDGEMENTS

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NOMENCLATURE

A	membrane area	m^2
C	concentration	mol m^{-3}
d	characteristic length: 4 × the cross-section divided by the wetted perimeter	m
D	diffusion coefficient	$m^2 s^{-1}$
E	enhancement according to Hatta theory	-
F	water flow	$m^3 s^{-1}$
Ha	Hatta number	-
J	flux through membrane	mol s^{-1}
k	mass transfer coefficient	m s^{-1}
k_r	pseudo-first-order reaction constant	s^{-1}
K_m	Michaelis-Menten constant	mol m^{-3}
m	partition coefficient (on mol m^{-3} basis)	-
v	liquid velocity	m s^{-1}
V_{max}	maximum volumetric activity of the cells	mol m^{-3} s^{-1}

Greek symbols

δ	membrane thickness	m
ε	porosity of the membrane	-
μ	viscosity	Pa s
ρ	density	kg m^{-3}
τ	tortuosity of the membrane	-
φ	flow into gas loop in Figure 3	mol s^{-1}

subscripts/superscripts

g	gas
in	flowing into gas loop in Figure 3
l	liquid
m	membrane
ov	overall

APPENDIX 1

Determination of the membrane mass transfer coefficient (k_m)

The mass transfer coefficient of the membrane was measured at 23 ± 1 °C in the 40 cm^2 membrane reactor shown in Figure 2. To exclude any mass transfer resistance in the liquid phase the transfer rates of water vapour and of two components with a high solubility in water, ethanol and propanol, were determined.

Liquid was pumped through one compartment of the reactor at a rate of 0.75×10^{-2} to 1.0×10^{-2} m s^{-1}. Through the other compartment air was blown at 0.54 to 5.4 m s^{-1}. In case of water transfer, tap water was used and the vapour pressure and temperature of the air leaving the reactor were measured with an Aqmel Dew Point Hygrometer. For the transfer of ethanol and 2-propanol solutions of 5 g l^{-1} of the alcohol in tap water were pumped over the membrane and the alcohol concentrations in the air leaving the reactor were determined in a manner similar to the determination of propene.

The liquid phase concentration of the diffusing component water was constant over the length of the reactor. The decrease in the alcohol concentrations in the liquid phase over the length of the reactor was less than 0.5%. Therefore in equation 5 the resistance in the liquid phase ($1/k_l$) can be neglected and a constant liquid phase concentration C_l can be assumed. Reducing the air velocity did not affect the transfer rate. Consequently the resistances in the gas phase ($1/(k_g \times m)$) in equation 5 can be neglected as well. The driving force for mass transfer ($C_l/m - C_g$) decreases over the length of the reactor since C_g increases. Therefore the logarithmic mean of the driving force was calculated. Assuming all resistance to be in the membrane, k_m values for the transfer of ethanol and 2-propanol were found as shown in Table 1.

Table A1- 1: Determination of the membrane mass transfer coefficients

Diffusing agent	k_m (m s^{-1})	D_g [a] (m^2 s^{-1})	k_m/D_g (m^{-1})
water	2.1 (± 0.2) × 10^{-2}	2.6 × 10^{-5}	8.1 (± 0.7) × 10^2
ethanol	1.2 (± 0.1) × 10^{-2}	1.35 × 10^{-5}	8.7 (± 0.8) × 10^2
2-propanol	8.9 (± 0.8) × 10^{-3}	0.99 × 10^{-5}	9.0 (± 0.8) × 10^2

[a] Calculated according to ref. 28.

In hydrophobic microporous membranes the pores are filled with gas and therefore the membrane mass transfer coefficient is a function of the gaseous diffusion coefficient and the porosity, thickness, and tortuosity of the membrane (22) according to the equation $k_m = (D_g \times \epsilon) / (\delta \times \tau)$. By dividing the mass transfer coefficient by the diffusion coefficient the expression k_m/D_g is obtained, which is independent of the diffusing agent. Interaction of the diffusing component with the membrane, i.e. Knudsen diffusion, is neglected. For the three compounds similar values for k_m/D_g were found (Table A1-1).

Kreulen et al. (14) determined the membrane resistance of the same type of membrane with several gas mixtures. For diffusion in N_2, which is quite similar to diffusion in air, they found values of k_m/D_g of 1.1×10^3 and 1.45×10^3 m^{-1} for NH_3 and mono-ethyl-amine respectively at 25°C. The lower value of k_m/D_g in our experiments can not be due to resistance in the liquid phase since we have also used pure water. The true k_m/D_g might be slightly higher due to a small resistance in the gas phase. If there is a substantial gas phase resistance, it is included in the membrane resistance determined experimentally.

APPENDIX 2

Description of the simulation program BIOSIM

With the simulation program BIOSIM reaction according to Michaelis Menten kinetics and simultaneous diffusion in a biolayer can be calculated numerically. Substrate consumption in a flat layer of biomass exhibiting Michaelis Menten kinetics can be described with the following general mass balance (25):

$$D_l \frac{\partial^2 C}{\partial x^2} = \frac{V_{max}\, C}{K_m + C} \qquad \text{(Eq. A2-1)}$$

The boundary conditions are:

$$\frac{dC}{dx} = 0 \qquad \text{at } x = L \text{ (or } x = x_r) \qquad \text{(Eq.A 2-2)}$$

$$C = C_g / m \qquad \text{at } x = 0 \text{ (no external resistance)} \qquad \text{(Eq. A2-3)}$$

with x = distance to the membrane
C = substrate concentration in biofilm
L = thickness of biofilm
x_r = the distance from the membrane where the substrate concentration approaches zero

This differential equation is solved using a Runge-Kutta algorithm. The detailed description of the computing scheme has previously been given by De Gooijer et al. (7).

External mass transfer resistance can optionally be taken into account. In case of gas-liquid transfer the concentration at the interface is calculated according to the film theory and equation (A2-3) converts into:

$$C = \frac{C_g}{m} = \frac{D_g \frac{dC}{dr_{x=0}}}{k_{external}} \qquad \text{at } x = 0 \qquad \text{(Eq. A2-4)}$$

In this case the gas phase concentration (C_g) in the reactor is constant because the gas is recirculated over the membrane at high speed.

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CHAPTER 4

Propene removal from synthetic waste gas using a hollow-fibre membrane bioreactor

Martine W. Reij and Sybe Hartmans

Summary: Hollow-fibre modules containing microporous membrane material were evaluated as bioreactors for waste gas treatment. The reactors were inoculated with the propene-utilizing strain *Xanthobacter* Py2, which formed a biofilm on the inner side of the fibres. The removal of the poorly soluble volatile propene from synthetic waste gas was monitored for up to 170 days. The maximum removal rates were 70 to 110 grams of propene per m^3 reactor per hour. A gas residence time of 80 s was required to remove 95 % of an initial propene concentration of 0.84 g m^{-3}. The presence of ammonium in the liquid medium resulted in the development of an additional population of nitrifying organisms. Therefore, nitrate was used as the source of nitrogen in later experiments. During long-term operation the propene removal rates gradually decreased. At low liquid velocities (1-5 cm s^{-1}) clogging of individual fibres with excess biomass was observed. Elevation of the liquid velocity in the fibres to 90 cm s^{-1} resulted in the formation of a dense biofilm and prevented clogging of the fibres. However, also at this high liquid velocity a gradual decrease in propene removal rate was observed. These results suggest that aging of biofilms is a very important factor in long-term operation of hollow-fibre bioreactors.

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INTRODUCTION

Biological methods have been used on an industrial scale to treat various exhaust gases with relatively low concentrations of biodegradable organic volatiles. Biodegradable volatiles are converted by microorganisms into mineral end-products (e.g. CO_2, H_20 etc.), and biomass. The oldest type of bioreactor for waste gas treatment is the biofilter, consisting of a bed of soil or compost with attached microorganisms, through which the gas is blown (14, 21).

Two other types of reactors are the trickle-bed reactor and the bioscrubber. In these reactors the contaminants are first transferred to the water phase. In a trickle-bed reactor the contaminants are then degraded by microorganisms that are present on the packing material in the packed bed and, in a bioscrubber, degradation is located in a separate tank containing activated sludge.

A major problem in biological waste gas treatment is the removal of low concentrations of volatiles with a poor water solubility (14). Volatiles with an air/water partition coefficient larger than 0.1 are considered to be poorly water soluble (13). In bioscrubbers and trickle-bed reactors a layer of water is present in between the gas phase and the biomass. For volatiles with a high air/water partition coefficient such an inert water layer forms a considerable mass transfer resistance (3).

A new membrane bioreactor with microporous hydrophobic membranes was designed for the removal of volatiles with a low solubility (9). Waste gas, supplying organic carbon and oxygen to the biofilm, flows along one side of the membrane (Figure 1A). The biofilm is located at the other side of the membrane and is supplied with minerals and water by the circulating liquid phase. The pores of the hydrophobic membrane are filled with gas. The biofilm is situated directly at the gas-liquid interface without a water layer between the gas and the biomass. Therefore, just like the biofilter, the membrane bioreactor is suitable for the removal of poorly soluble pollutants. In a biofilter, however, the humidity and the pH can not be controlled due to the absence of a circulating water phase. In a membrane reactor the water phase can remove degradation products, such as HCl, and humidifies the biomass without forming a barrier for mass transfer. Hydrophobic membranes have been used by Hinz et al. (10) in their membrane bioreactor for the elimination of nitrogen monoxide from air. Silicon tubes have also been tested in bioreactors for waste gas treatment (1, 6).

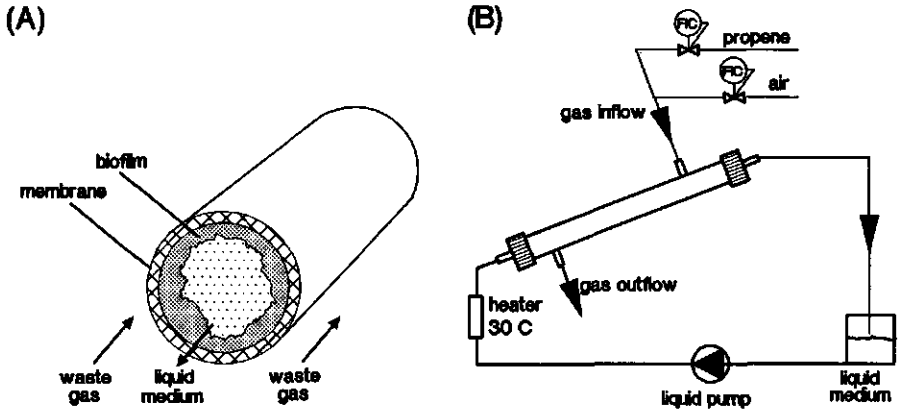

Figure 1. Schematic view of (A) one hollow-fibre with biofilm and (B) a complete setup with membrane module and gas and liquid flows.

Propene (air/water partition coefficient = 10) was chosen as a model pollutant to test the performance of the membrane bioreactor for the treatment of waste gases containing poorly water soluble pollutants. In a previous study (16) the mass transfer properties of flat sheet membrane reactors were determined. This study focusses on the long-term operational stability of hollow-fibre membrane bioreactors. Hollow-fibre modules (Figure 1B) have a considerably larger gas-water interface than the flat sheet bioreactors used previously, allowing higher volumetric removal rates.

MATERIALS AND METHODS

Microorganism

Xanthobacter Py2 was isolated with propene as sole source of carbon (20). Membrane reactors were inoculated with cultures of *X.* Py2 grown at 30°C in 5-l Erlenmeyer flasks containing 0.5 l mineral salts medium (pH 7.0) and 4 % (vol vol^{-1}) propene in air as the carbon source. After inoculation the reactors were operated in a non-sterile way.

Chemicals and media

Propene (99.99% pure) was purchased from Hoek Loos (Schiedam, The Netherlands). Propene was mixed with ambient air using Thermal Mass Flow Controllers (Brooks Instrument, Veenendaal, The Netherlands). Mineral salts medium was prepared as described by Hartmans et al. (8) and contained 2 g l^{-1} $(NH_4)_2SO_4$. Since nitrification occurred during

prolonged operation of the bioreactors, in later experiments $(NH_4)_2SO_4$ was replaced by 3.0 g l^{-1} KNO_3 and 0.7 g l^{-1} Na_2SO_4. All chemicals were reagent grade.

Hollow-fibre membrane bioreactor

The layout of the hollow-fibre membrane bioreactor is shown in Figure 1B. The hollow-fibre membrane modules (type MD 020 CP 2N, Microdyn, Wuppertal, Germany) contained 40 hydrophobic polypropylene fibres with a length of 500 mm, an inner diameter of 1.8 mm, and a pore size of 0.2 µm. The internal volume of the modules was 157 ml and the total membrane-liquid interface was 0.1 m^2 (637 m^2 m^{-3}). Air containing propene was supplied at a rate of 275 ml min^{-1}, corresponding to a gas residence time of 7.4 s. The module was mounted sloping with an angle of 20° to allow condensate to be removed quickly from the gas phase. Liquid was circulated through the fibres with a velocity ranging from 1 to 5 cm s^{-1}, using a Watson and Marlow 502S pump. Several modules, however, were operated at a liquid velocity of 90 cm s^{-1}, using a Verder 2035 pump. The total liquid volume in the system was 1.25 l. The liquid entering the membrane module was kept at 30 (± 1) °C. In the well mixed systems with a liquid velocity of 90 cm s^{-1}, the pH and the oxygen tension, pO_2, were measured at regular intervals in the buffer vessel.

Membrane bioreactor operation

On day 0 the liquid phase was inoculated with a propene-grown culture of *Xanthobacter* Py2 to an optical density of 0.3 at 660 nm. From this moment on the reactor was operated as an open system, i.e. in a non-sterile way. Water loss due to evaporation in the hollow fibres was replenished on a regular basis with demineralised water. Periodically, as indicated in the individual experiments, 0.5 l of the liquid phase were removed and replaced with fresh mineral salts medium to supply nutrients. After each experiment the hollow-fibre modules were opened and the individual fibres were examined for clogging.

Sampling and activity assays

The gas flows going in and out of the bioreactors were sampled every 2 h and analysed for propene and CO_2 using an on-line CP 2001 gas-analyser (Chrompack, Middelburg, The Netherlands) equipped with a Hayesep A column (25 cm) at 70 °C and a thermal conductivity detector. During the experiments in which the propene concentration or gas residence time were varied, the propene concentrations were determined manually. Detachable glas containers (35 ml) were placed in line with the ingoing and outgoing gas flows and 100 µl gas samples from these containers were injected into a gas chromatograph equipped with a Porapak R column (100-120 mesh) at 210°C and a flame-ionization detector. All values are the average of at least three separate determinations.

The concentrations of ammonium, nitrate, and nitrite in the liquid phase were measured using an auto-analysis system (Skalar 5100) (7).

The propene oxidation activities in the liquid phase were determined by incubating 5-ml samples of the liquid phase with 0.3 % (vol vol^{-1}) propene in air in serum flasks at 30°C under vigorous shaking, as described previously (16).

RESULTS

Continuous removal of propene from air

Biofilters used for waste gas treatment are usually operated with a gas load in the range of 100 to 200 m^3 gas per m^3 reactor per hour (21). We selected a volumetric load of 106 m^3 gas per m^3 reactor per hour to test the membrane bioreactor. This load corresponds to a gas residence time of 7.4 s. A relatively high propene concentration of 6 g m^{-3} was chosen, as we first wanted to study the reactor performance under conditions that allowed adequate growth.

Figure 2 shows both the propene removal rates and the CO_2 production rates of the 0.1 m^2 hollow-fibre membrane bioreactor (MBR). The data represent daily averages of ≈12 automated measurements. Approximately 10 days after start-up the efficiency of the reactor increased sharply, but it decreased again from day 17 onwards. In a similar experiment, with a lower concentration of 0.92 g m^{-3} propene in the gas phase, the propene removal rate also decreased to a very low value after two months of operation. Neither the addition of fresh mineral salts medium, nor the addition of propene-grown cells could restore the propene conversion (data not shown).

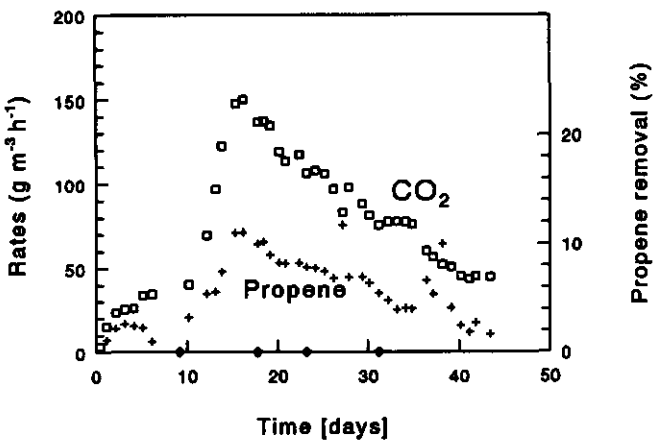

Figure 2. Propene removal (+), and CO_2 production (□) rates in a hollow-fibre membrane reactor with a propene concentration of 6.0 g m^{-3} in the ingoing gas flow. Propene removal is indicated as g m^{-3} h^{-1} (*left*) and as a percentage (*right*). On days indicated with (♦) the liquid medium was refreshed. The propene load was 624 g m^{-3} h^{-1}.

The total amount of propene that had been degraded from day 0 until day 44 was calculated to correspond with 3.8 g of carbon. After subtraction of the amount of carbon recovered as CO_2, the total biomass production can be estimated to be 3.1 g (C content = 45%). In the liquid phase only 0.6 g of dry biomass were recovered, suggesting that 2.5 g of dry weight (50 kg of biomass per m^3) were present in the fibres. For comparison, the density of a biofilm of *Xanthobacter autotrophicus* GJ10 was found to be on average 60 kg m^{-3} (4). When the reactor was dismantled, the liquid phase indeed appeared to flow through only four of the 40 fibres, indicating that most of the fibres were clogged with biomass.

Effect of liquid velocity on the stability of propene consumption

The liquid velocity was increased to see if this would prevent deterioration of the reactor performance. The volumetric gas load was the same as in the previous experiment, but the concentration of propene was slightly lower at 4.0 g m^{-3}. Two modules were operated simultaneously. In one module the liquid velocity in the empty fibres was 90 cm s^{-1} ("fast") and in the second module the liquid velocity was 3.4 cm s^{-1} ("slow"), similar to the previous experiment.

Figure 3 shows the propene removal of these modules during 170 days. Maximum removal rates of 110 and 70 g m^{-3} h^{-1} were reached in the "fast" and "slow" modules, respectively, after 25 days. As the data are the daily averages of 12 measurements, and the standard deviation for propene measurement was only 2 %, the considerable variations in propene removal rates were not due to sampling errors. From day 65 onwards, when the liquid phase was refreshed less frequently, the variation in the propene removal rates decreased. During this period the removal rates gradually decreased from 50 to 35 g m^{-3} h^{-1} in the "fast" module and from 50 to 25 g m^{-3} h^{-1} in the "slow" module.

After dismantling the reactor several fibres of the "slow" module appeared to be clogged with biomass. In the "fast" reactor, none of the fibres were clogged with biomass and most fibres appeared to contain an evenly distributed biofilm. A cross-section of the "fast" module is shown on the front cover of this thesis.

Effect of nitrification on pH, pO_2, and propene consumption

During the experiments shown in Figure 3 a drop in pH was observed after the addition of fresh mineral salts medium, sometimes (days 29, 42 and 62) coinciding with a temporary decrease in the propene removal rate. Nitrification was likely to be responsible since the addition of mineral salts medium without ammonium did not trigger these effects (data not shown). A population of nitrifiers could develop since the system was operated in a non-sterile way.

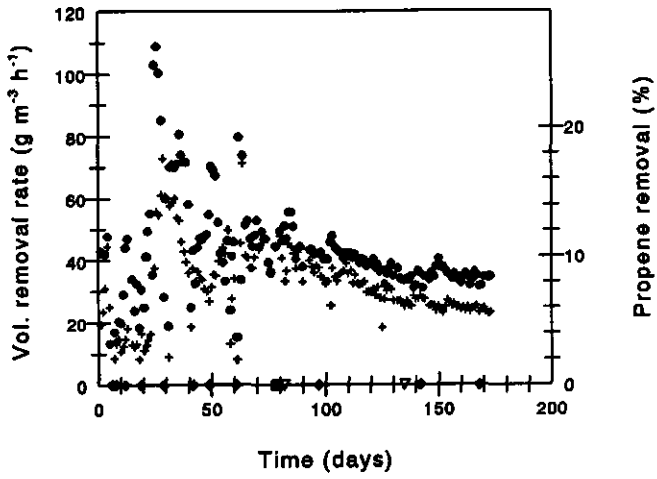

Figure 3. Volumetric propene removal rates and the percentages of propene removed in hollow-fibre membrane modules with ammonium as nitrogen source. The liquid velocities in the fibres were 3.4 cm s^{-1} (+) and 90 cm s^{-1} (●). The concentration of propene in the ingoing air was 4.0 g m^{-3} and the propene load was 424 g m^{-3} h^{-1}. As indicated on the x-axis 0.5 l liquid were replaced with mineral salts medium containing 30 mM ammonium (♦), no nitrogen source (■), or 30 mM nitrate (▽).

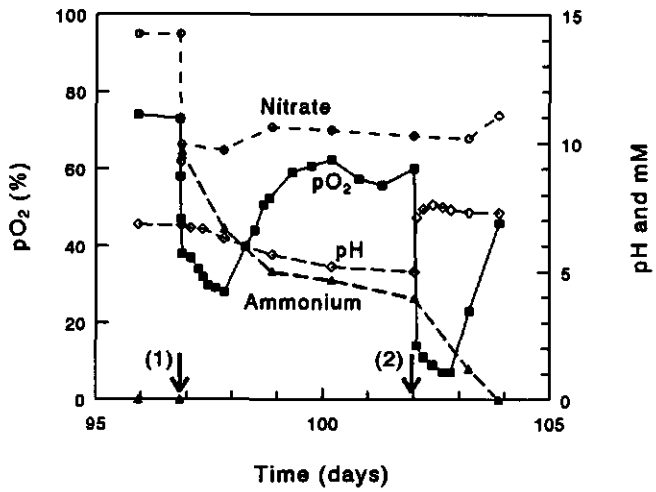

Figure 4. Effect of the addition of ammonium sulphate on the pH (◇), the oxygen tension (■), and the ammonium (▲) and nitrate (○) concentrations. On day 97 (1) 0.5 l liquid was replaced with mineral salts medium containing 30 mM ammonium sulphate. On day 102 (2) the pH was adjusted manually to pH 7.0.

To confirm nitrification, mineral salts medium containing ammonium was added again on day 97 and the pH, the pO_2 and the nitrogen concentrations in the liquid medium of the well mixed "fast" module were measured to check for the presence of nitrifiers (Fig. 4). On day 102 approximately 40% of the ammonium was still present. On that day the pH, which had dropped to 5.3, was adjusted manually to 7.0. Subsequently a second drop in pO_2 was observed, probably due to the renewed activity of the nitrifiers that had been inhibited by the low pH. The reduced pO_2 did not cause a temporary decrease of the propene removal rate, as had been observed on days 29, 42 and 62 in the same reactor. The nitrate level remained relatively constant at 10 mM, a maximum concentration of 0.2 mM of the intermediate nitrite was found in the liquid, and after 7 days all ammonium had been consumed.

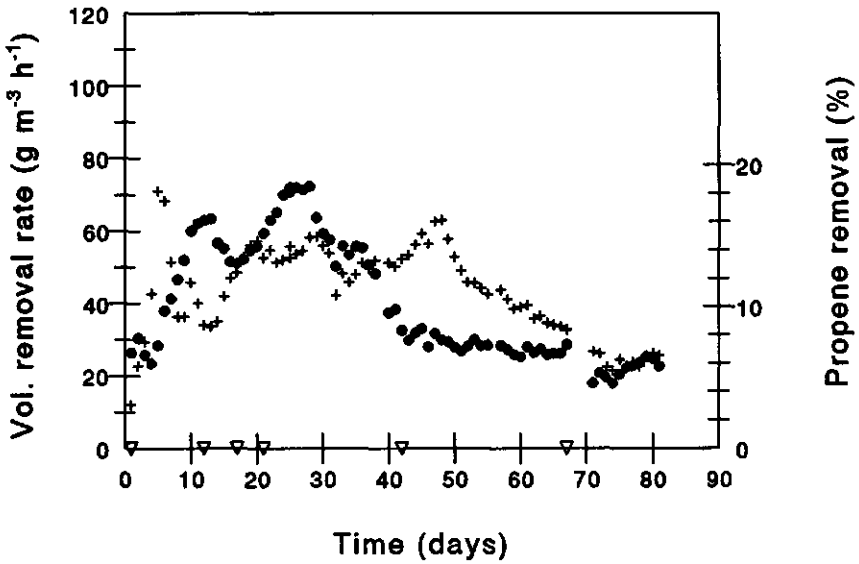

Figure 5. Volumetric propene removal rates and the percentages of propene removed in hollow-fibre membrane modules with nitrate as nitrogen source. The liquid velocities in the fibres were 3.4 cm s^{-1} (+) and 90 cm s^{-1} (●). The concentration of propene in the ingoing air was 4.0 g m^{-3} and the propene load was 424 g m^{-3} h^{-1}. On dates indicated with (▽), 0.5 l liquid were replaced with mineral salts medium containing 30 mM nitrate.

Nitrate as source of nitrogen

To prevent nitrification and thereby possibly improve the stability of propene removal, the ammonium in the mineral salts medium was replaced by nitrate in the next experiment. The growth rates of *Xanthobacter* Py2 on propene in serum flasks were found to be the same with both mineral salts media (data not shown). Once again, as in the experiment using ammonium as nitrogen source, one module ("fast") was operated at a liquid velocity of 90 cm s^{-1} and the other ("slow") at 3.4 cm s^{-1}. Figure 5 shows the propene removal rates in these modules. The pH and pO_2 in the liquid of the "fast" module were stable during the entire experiment (data not shown). The variations in the propene removal rates were considerably less than the, sometimes acute, variations observed in the modules in which ammonium was used as the nitrogen source (Fig. 3). In both the "slow" and the "fast" modules maximum removal rates of 60 to 70 g m^{-3} h^{-1} were observed. After 40 days the removal rate in the "fast" module dropped relatively quickly to 30 g m^{-3} h^{-1}. After 70 days the removal rate in the "slow" reactor had decreased to the same level.

CO_2-production

The carbon recovered as CO_2 in the gas phase was calculated as the percentage of the propene consumed. In the "fast" reactors the CO_2 yields were 78 and 86 %, being significantly higher than in the corresponding "slow" modules (70 and 74 %). With nitrate as nitrogen source the CO_2-yields were 74 and 86 %, against 70 and 78 % with ammonium. The reason could be the assimilation of nitrate, which requires energy resulting in a lower biomass yield and higher CO_2 yield. However, the lower CO_2 production in the presence of ammonium could also be a result of simultaneous CO_2 consumption by the ammonium oxidizers. It should be noted that in all experiments the CO_2 yields were significantly higher than the 54±2 %, measured in continuous liquid cultures (17).

Variation of propene concentration and gas residence time

During the experiment in which nitrate was used as nitrogen source the propene removal rates were relatively stable around 60 g m^{-3} h^{-1} between day 27 and 41, especially in the "slow" module. During this period the inlet propene concentration and gas residence time were varied to study the performance of the reactor over wide a range of conditions. Each measurement lasted ≈3 hours and in between the measurements the standard conditions were applied.

Between days 27 an 31 the concentration of propene in the gas phase was varied at a constant gas flow rate of 275 ml min^{-1}. Figure 6 shows the volumetric propene removal rates and the fluxes as a function of the propene concentration in air. The

propene concentration in this figure is the calculated logarithmic mean of the propene concentrations at the inlet and outlet of the reactor. The maximum propene conversion was 32%. The "slow" module was slightly more efficient than the "fast" module at low concentrations of propene.

In a previous study the propene fluxes into a biofilm of *Xanthobacter* Py2 growing on a flat sheet membrane were measured and simulated (16). For comparison the simulated data and the results from both reactor configurations are presented in grams per m^2 membrane per hour. In the simulations maximum biofilm activities of 4.5 and 9.1 kg propene per m^3 biofilm per hour were assumed.

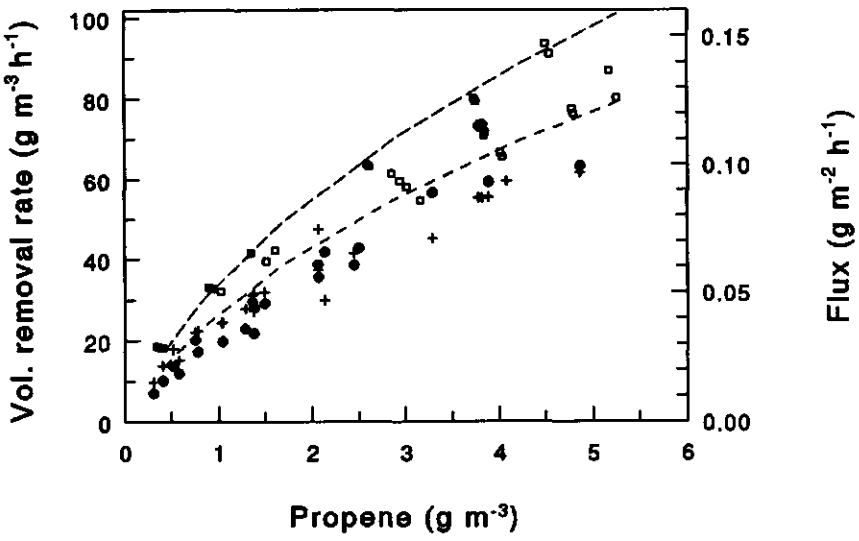

Figure 6. Propene removal rates (g m^{-3} h^{-1}) and propene fluxes (g m^{-2} h^{-1}) as a function of the propene concentration for liquid velocities of 3.4 cm s^{-1} (+) and 90 cm s^{-1} (●). The correlation between the volumetric removal rate and the flux is the specific membrane area in the hollow-fibre module (637 m^2 m^{-3}). The air flow rates were constant at 275 ml min^{-1}. These data were measured during the experiment shown in Figure 5, between days 27 and 31. The fluxes (□) obtained with a biofilm of *Xanthobacter* Py2 growing in a flat sheet membrane bioreactor (16) are shown for comparison, in this case only in g m^{-2} h^{-1}. The dotted lines represent simulated values assuming maximum biofilm activities of 4.5 (·····) and 9.1 (----) kg propene per m^3 biofilm per hour (g m^{-2} h^{-1} only).

Between days 33 and 41 the propene conversion in the "slow" reactor was measured as a function of the gas residence time for initial propene concentrations of 0.84 g m^{-3} and 4.0 g m^{-3}. As can be seen in Figure 7, propene conversion depends both on the gas residence time and on the propene concentration in the gas inlet. At a gas residence time of 80 s and an inlet concentration 0.84 g m^{-3}, 95% propene removal was obtained. With an inlet propene concentration of 4.0 g m^{-3} 80% removal was realized with the same residence time.

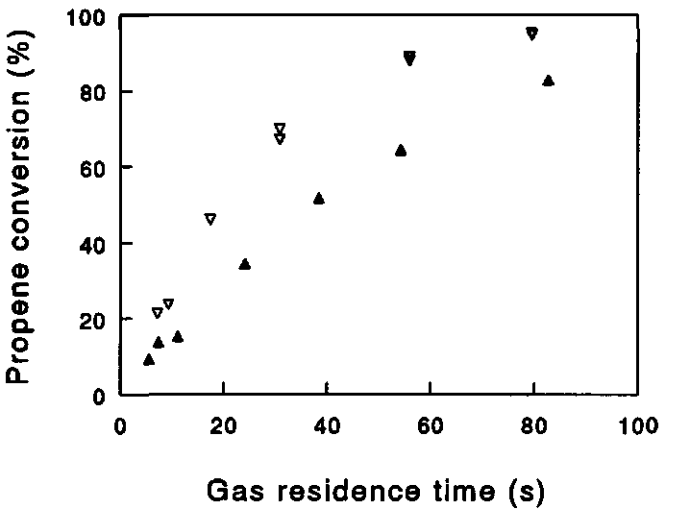

Figure 7. The effect of the gas residence time on the propene conversion in a membrane reactor with a liquid velocity of 3.4 cm s^{-1} and nitrate as nitrogen source. The concentrations of propene in the air entering the reactor were (▽) 0.84 g m^{-3} and (▲) 4.0 g m^{-3}. Data were measured during the experiment shown in Figure 5, between days 33 and 41.

DISCUSSION

Propene removal rates

During continuous operation at a residence time of 7.4 s the propene conversion was maximally 20 %. Nevertheless, much higher conversion rates are feasible if the residence time of the gas in the hollow-fibre reactor is increased. With a gas residence time of 80 seconds an inlet concentration of 840 mg m^{-3} of propene could be reduced by 95 %. For higher propene concentrations longer residence times are required to obtain the same reduction.

In a previous study the propene fluxes into a biofilm of *Xanthobacter* Py2 growing on a flat sheet membrane were measured and simulated (16). The removal rates (in grams per m^2 per hour) in the hollow-fibre system were approximately 80 to 90% of the range of values measured in the flat sheet reactor, exept for three data points, which were measured on day 27, when the module was operating very efficiently. Thus, in a hollow-fibre module the same flux (in grams per m^2 membrane per hour) as in a flat sheet reactor can be realized for a limited period of time. However, due to the high specific area in the hollow-fibre reactor (637 m^2 m^{-3}), higher volumetric removal rates are attainable with this reactor configuration.

Comparison with other reactor types

We have only found a few reports in the literature on the removal of components with a low water solubility. Sly et al. (18) used a trickle-bed reactor to remove methane from coal mine ventilation gas and found a linear relationship between concentration and removal rate. The data can not be compared to the propene removal data because differences in diffusion and partition coefficients, and gas residence times.

De Heijder et al. (3) measured the removal of ethene, which has an air-water partition coefficient and a diffusion coefficient similar to that of propene. Ethene removal was measured in a packed bed of granular activated carbon with inlet concentrations of 0.14 to 0.9 grams ethene per m^3. With the higher ethene concentrations tested, the volumetric ethene removal rates were similar to the propene removal rates measured in the hollow-fibre modules. At lower concentrations the dry bed of activated carbon was slightly more efficient in the removal of ethene than the hollow-fibre modules were in the removal of propene.

Nitrification

Due to the regular addition of ammonium to the liquid phase a population of ammonium oxidizers apparently developed in the hollow-fibre reactor, making it

suitable for the removal of ammonia from air. However, the presence of nitrifiers might be responsible for the observed unstable propene conversion and complicated the study of propene removal in several ways. In the first place the nitrifiers consumed oxygen and produced acid (Figure 4). The lowered pO_2 and pH, as well as small amounts of nitrite produced, might inhibit propene oxidation. On the other hand, ammonium oxidizing microorganisms possessing non-specific monooxygenases (2), might be responsible for part of the propene oxidation in the reactor.

To simplify the system it was therefore decided to replace ammonium with nitrate in the liquid phase, although some *Xanthobacter* spp. have been reported to supply their nitrogen demands by nitrogen fixation (22). With nitrate the propene removal (Figure 5) varied to a lesser extent than with ammonium (Figure 3). However, the propene removal rate still decreased irreversibly within 70 days. Therefore, apart from the presence of a destabilizing population of ammonium oxidizers, there must be another cause for the decrease in performance of the biofilm reactor.

Production of CO_2 and biomass

During the first experiment most of the fibres seemed to be filled up completely with biomass. This observation was corroborated by the estimated average biomass density of 50 kg m^{-3}. Problems due to the formation of excess biomass have also been observed with other applications. In membrane bioreactors for wastewater treatment the performance was reduced due to excessive biofilm growth (5) and a daily removal of the biomass was required (15).The highest ratio of CO_2 over propene (and the lowest biomass yield) was observed with nitrate as the nitrogen source and at a high liquid velocity.

Clogging of the fibres with biomass could be prevented by a high liquid velocity. With both nitrogen sources tested, an evenly distributed biofilm was found to be attached to inside of the fibres in the "fast" modules, while the fibres in the corresponding "slow" reactors were largely filled with clumps of biomass. Although clogging of the fibres did not occur at the high liquid velocity, the performance of these modules also decreased in time. Apparently, clogging of the reactor is not the only factor resulting in a reduction of the reactor performance. Possibly the diffusion of nutrients from the liquid phase to the active part of the biofilm close to the membrane, could be limited by the thick layer of biomass. However, replacement of the liquid phase with mineral salts media with or without nitrogen source, did not influence the propene removal rate, indicating that the nitrogen supply was not crucial.

Biofilm stability

Another possible cause for a decreased performance of the membrane reactor is the accumulation of dead cells close to the membrane or the penetration of water into the microporous membrane if the hydrophobic membrane is wetted. Both processes result in the formation of an aqueous layer hindering transfer of the poorly water soluble propene.

Before hollow-fibre membrane reactors can be used for waste gas cleaning practice, the problems associated with the instable biofilm performance should be solved. Regular removal of the biomass by backwashing (15) or (bio)chemical treatment (5) might be used to regenerate the membrane bioreactor. Furthermore, detailed information on the processes in the biofilm is required. Information about the localization of the active biomass could be obtained using ATP measurements (12) or probes assessing cell viability (11). Microelectrodes could provide information on the oxygen and nitrogen profiles in the biofilm (19).

For the time being dry biobeds (3) or biofilters (13) seem more suitable for the removal of volatiles with a low water solubility. However, an advantage of the membrane reactor over the biofilter and the dry biobed is the presence of the water phase in the membrane bioreactor. In this way, inhibitory degradation products, such as HCl formed during the degradation of chlorinated volatiles, may be removed.

ACKNOWLEDGEMENTS

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CHAPTER 5

Biofiltration of air containing low concentrations of propene using a membrane bioreactor

Marline W. Reij, Erik K. Hamann, and Sybe Hartmans

Summary: Volatiles with a low water solubility are difficult to remove from air by biofiltration, especially when present in low concentrations, because the driving force for mass transfer into the aqueous phase is very small. We have used microporous hydrophobic membrane material as a support for biofilms that remove the poorly soluble propene from air. Two bacterial strains were compared and the faster growing strain, *Xanthobacter* Py2, was selected to study biofilm formation and reactor performance at propene concentrations in the range encountered in off-gas treatment. With a concentration of 200-350 ppm of propene in the gas phase, a 20 days start-up period was required for the formation of a biofilm on the membrane. Once the biofilm had been established, continuous propene removal was tested over a range of concentrations. The amount of active biomass adapted to the amount of propene available. When the membrane bioreactor was operated with propene concentrations as low as 9 to 30 ppm in the gas phase, the propene removal rate was stable for several weeks, without the supply of any other source of carbon or energy to the microbial population.

INTRODUCTION

Many human activities result in the contamination of air with organic volatiles. As public concern about air-pollution is growing, stringent regulations on off-gases have been enforced in several industrial countries. Table 1 shows the maximum concentrations of pollutants allowed in off-gas in The Netherlands. The maximum allowable concentrations primarily depend on the toxicity of the pollutants, but also on the total mass flow and the overall composition of the off-gas (16). The various individual pollutants may have to be reduced to lower concentrations than those indicated in Table 1, if they are present as a mixture of pollutants.

Since the early 1980s biological methods have increasingly been used in Germany and The Netherlands to remove various volatile contaminants emitted from industrial facilities (10). The type of reactor most commonly used is the biofilter, consisting of biologically active material, primarily compost, peat or soil. Microorganisms present in the biofilter use the contaminants in the waste gas as growth substrates.

As microorganisms require a relatively high water activity to be physiologically active, the actual degradation is situated in the water phase. Both the contaminant and oxygen, required for its oxidation, have to be transferred from the gas phase to the water phase. The actual concentration available to the microorganisms depends on the air/water partition coefficient (m) of the contaminant.

$$m = \frac{\text{concentration in the gas phase (mol m}^{-3}\text{)}}{\text{concentration in the water phase (mol m}^{-3}\text{)}}$$

Table 1 shows the aqueous concentrations that correspond to the maximum allowable concentrations in air. For volatiles with a low water solubility (high m) the corresponding aqueous concentrations are extremely low. As a consequence the driving force for mass transfer over the aqueous layer, surrounding the microorganisms, is small and thus these volatiles are difficult to remove from air.

Membrane bioreactor

For the continuous removal of poorly soluble contaminants from air a bioreactor with hydrophobic microporous membranes was chosen because of its excellent mass transfer properties (14). In this reactor the membrane forms the interface between the gas phase and the liquid phase (Figure 1). The pores of this hydrophobic microporous membrane are gas-filled and thus diffusion through the membrane is essentially diffusion in gas.

Furthermore a large gas-liquid interface can be created in e.g. hollow fibre modules and toxic degradation products can be removed by the water phase. Microorganisms in the liquid phase attach to the membrane and form a biofilm (Figure 1). This biofilm is supplied with minerals and water from the liquid phase while organic pollutants and oxygen are supplied by diffusion through the membrane.

Table 1. Maximum concentrations of various volatiles allowed in off-gas in The Netherlands (16).

Contaminant	maximum concentration in off-gas ($mg\ m^{-3}$)		(ppm)	air/water partition coeff.[a] (m) at 25°C (-)	corresponding concentration in water (μM)
aliphatic hydrocarbons					
total	150		-	NA	NA
ethene	150		135	8.8	0.62
propene	150	[b]	90	8.6	0.42
methane	150	[b]	236	27	0.35
hexane	150	[b]	44	69	0.026
aromatic hydrocarbons					
total	100		-	NA	NA
benzene	5		1.6	0.22	0.29
phenol	20		5.4	0.000019	1.1×10^{4}
styrene	100		24	0.13	7.4
toluene	100		27	0.26	4.2
various volatiles					
1,2-epoxypropane	5		2.2	0.0033	26
ethylene oxide	1		0.6	0.0056	4.1
acetone	150		65	0.0016	1.6×10^{3}
ethanol	150		82	0.00021	1.6×10^{3}

[a] References 1 and 12.

[b] Equal to the total concentration of aliphatics allowed.

Microorganisms growing with propene

We selected the unsaturated hydrocarbon propene as a model pollutant to study the removal of poorly soluble contaminants present in off-gas in low concentrations. Two bacterial strains are available that grow with propene as the sole source of carbon and energy, *Mycobacterium* Py1 and *Xanthobacter* Py2.

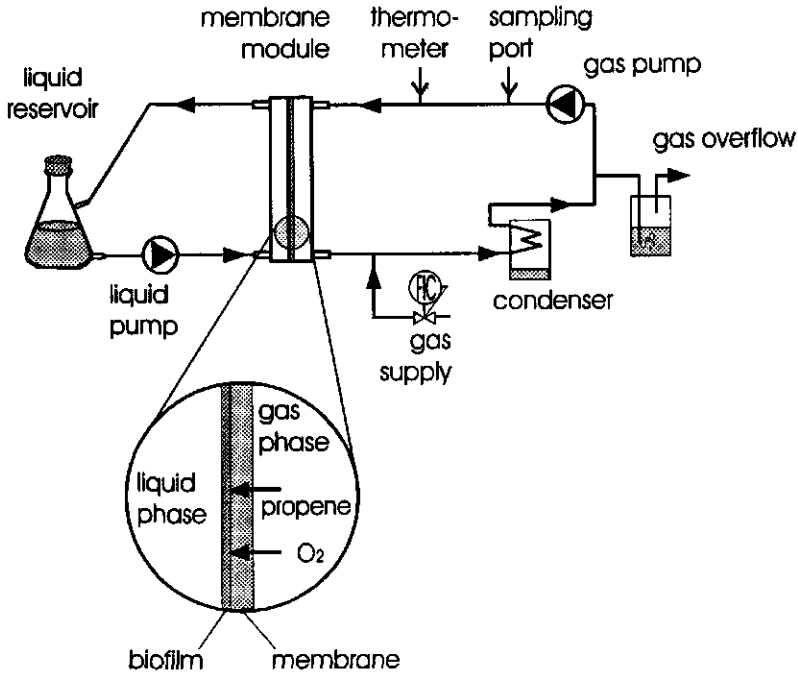

Figure 1. Set-up for biofilm formation and continuous propene degradation in a bioreactor with a 40-cm^2 microporous hydrophobic membrane. The magnification is a schematic representation of the membrane module with the membrane separating the gas- and liquid-compartments.

For the cultivation of these strains propene is usually supplied at a concentration of 1 to 5 percent in air (17- 84 g m^{-3}). In waste gas treatment, however, the concentration of aliphatic hydrocarbons must be reduced to less than 150 mg m^{-3} to comply with the emission regulations (Table 1). This implies that the microorganisms have to be cultivated at concentrations that are two to three orders of magnitude lower than usually applied under laboratory conditions.

In nature poorly soluble volatiles are known to be degraded in soil down to very low concentrations. Ethene e.g. is reduced in oxic soil to less than 0.1 ppm (7) and methane oxidizers present in soil can degrade methane to less than the ambient concentration of ≈1.8 ppm (2). However, the microbial capacity to degrade such low concentrations has scarcely been examined in bioreactors.

Objectives of this study

Here we describe a membrane bioreactor for the treatment of air contaminated with the poorly soluble propene. We focus on the continuous microbial degradation of very low concentrations of propene, without the supply of other sources of carbon or energy to the microbial population.

MATERIALS AND METHODS

Source and cultivation of microorganisms.

Mycobacterium Py1 (4) and *Xanthobacter* Py2 (18) were isolated with propene as the carbon source. Both strains were grown continuously on propene in a 1.5-l fermentor containing mineral salts medium. This medium contained per liter: 2 g $(NH_4)_2SO_4$; 1.55 g K_2HPO_4; 0.85 g $NaH_2PO_4.H_2O$; 100 mg $MgCl_2.6H_2O$; 5 mg $FeSO_4.7H_2O$; 2 mg $ZnSO_4.7H_2O$; 1 mg $CaCl_2.2H_2O$; 1 mg $MnCl_2.4H_2O$; 0.4 mg $CoCl_2.6H_2O$; 0.2 mg $CuSO_4.5H_2O$; 0.2 mg $Na_2MoO_4.2H_2O$; 10 mg EDTA. The dilution rates were 0.02 and 0.04 h^{-1} for Py1 and Py2, respectively. The impeller speed was 550 min^{-1}, the temperature was 30°C, and the pH was kept constant at pH 7.0 by titration with 2 M sodium hydroxide. 1.25 % (vol vol^{-1}) propene in air was supplied at a rate of 100 ml min^{-1}. This propene concentration is well below the minimal concentration for explosion, which is 2.4% (vol vol^{-1}) (11).

For the determination of the kinetic parameters propene-grown cells were harvested by centrifugation, washed in washing buffer (50 mM potassium phosphate buffer, pH 7.0), and resuspended in the same buffer.

For the induction experiments both strains were cultivated in 5-l Erlenmeyers containing 3 g l^{-1} sodium succinate and 0.5 l of mineral salts medium of which the amount of phosphate buffer was doubled. The cells were harvested in the mid-log phase by centrifugation, washed with mineral salts medium, and resuspended in the same medium. *X.*Py2 harvested in the mid-log phase contained slime and could not be washed properly. Slime-formation is characteristic for the genus *Xanthobacter* (21) when grown with excess carbon.

Determination of kinetic parameters for propene degradation

Kinetic parameters of propene degradation were determined by adding 1 ml of washed propene-grown cells to a 130-ml serum flask containing 4 ml of washing buffer and a known concentration of propene. The propene degradation rate was then determined in ≈ 30 min. For each initial propene concentration two cell densities were studied to test if the specific rates were equal for both the cell densities. If so, diffusion limitation could be ruled out. If the specific degradation rate was lower at the higher cell density, the experiment was repeated using lower cell densities.

Induction of the enzymes required for propene degradation

1 ml of washed succinate-grown cells (10-20 mg dry weight) were injected into a 130-ml serum bottle containing 9 ml of mineral salts medium and a known amount of propene and

the propene concentration was monitored in time. Control bottles were amended with 50 μg ml^{-1} chloramphenicol which inhibits *de novo* protein synthesis in bacterial cells.

Propene assay

The propene concentrations in air were determined by analyzing 100-μl headspace samples on a gas chromatograph equipped with a 20% Tween column (Chromosorb W AW 80-100; Chrompack, Middelburg, The Netherlands) and a flame-ionization detector. The column temperature was 110°C and the detection limit was ≈ 0.5 ppm.

Biomass dry weight

Biomass dry weights were determined by centrifuging 50-ml samples, resuspending in demineralized water, and drying at 108°C for 1 to 2 days.

Membrane bioreactor

The membrane material used in all experiments was hydrophobic polypropylene Accurel membrane, type 1E-PP, provided by Enka AG (Wuppertal, FRG). The porosity was 70-75 %, average pore diameter 0.1 μm and thickness 75-110 μm as stated by the supplier. This flat membrane was clamped between two identical halves of a perspex reactor and the membrane separated the reactor into two compartments. Through one compartment liquid was circulated while through the other compartment gas was blown. The effective membrane area was 40 cm^2 and the volume of each compartment was 8 cm^3.

The set-up in Figure 1 was built to measure the flux of propene through the membrane. The liquid phase, containing 400 ml of mineral salts medium, was circulated at a rate of 3 ml min^{-1}. The gas phase (volume = 400 ml) was circulated at a rate of ≈ 1 l min by a gas pump (KNF Neuberger, Freiburg, FRG) ensuring proper mixing of the gas phase and a constant concentration of propene over the length of the biofilm. A mixture of propene in air was supplied to the gas loop at a rate of 0.1 to 3 ml min^{-1} using a thermal mass flow controller (Brooks Instrument, Veenendaal, The Netherlands). The gas-loop was fitted with a thermometer and a gas-sampling port and excess air left the loop through the overflow. The whole set-up was placed in an isothermal chamber kept at 30°C, except for the condenser, the gas overflow, and the gas pump.

Bioreactor operation

On day 0 the sterilized membrane bioreactor was inoculated by circulating a propene-grown suspension of *Xanthobacter* Py2 over the membrane. The supply of propene into the gas loop was 9×10^{-9} mol s^{-1}. The propene concentration in the loop was monitored daily to determine the propene consumption of the developing biofilm. All data have been corrected for leakage. After a biofilm had developed, the supply of propene into the gas loop was changed daily and the resulting steady-state propene concentrations in the gas loop were measured after 16 to 24 hours. From the combination of supply rate and measured concentration, the propene consumption rate was calculated.

For the development of a biofilm at a low concentration of propene, the flux of propene into the gas loop was decreased to 1.2×10^{-9} mol s^{-1}. The biofilm was grown and monitored

analogous to the biofilm at high concentration. From day 27 to 42 [period (2)] the supply of propene was changed daily and the steady-state propene concentrations and consumption rates were determined. From day 42 to day 50 [period (3)] the supply of propene was reduced to 8×10^{-11} mol s^{-1}. Subsequently, the biofilm was allowed to recover at a supply of 1.2×10^{-9} mol s^{-1} during period (4) and the minimal supply was repeated from day 58 onwards [period (5)].

In addition to the frequent steady-state measurements the consumption rates were measured dynamically as a function of concentration on days 22, 26, 61, and 76. During these dynamic measurements both the supply and the overflow of the gas loop were closed, propene was added to the gas loop, and the concentration of propene was sampled regularly over a period of several hours in the closed system. Data have been corrected for leakage and loss of propene due to sampling.

RESULTS AND DISCUSSION

Biofilm formation and propene removal at high concentrations

Before we studied the feasibility of removing low concentrations of propene from air, we tested both *Xanthobacter* Py2 and *Mycobacterium* Py1 for their ability to form a biofilm at a high concentration of propene. Each strain was inoculated in a flat sheet membrane bioreactor. Propene was supplied at a rate of 5.8×10^{-8} mol s^{-1} resulting in gas phase concentrations of $\approx 2.3 \times 10^3$ ppm.

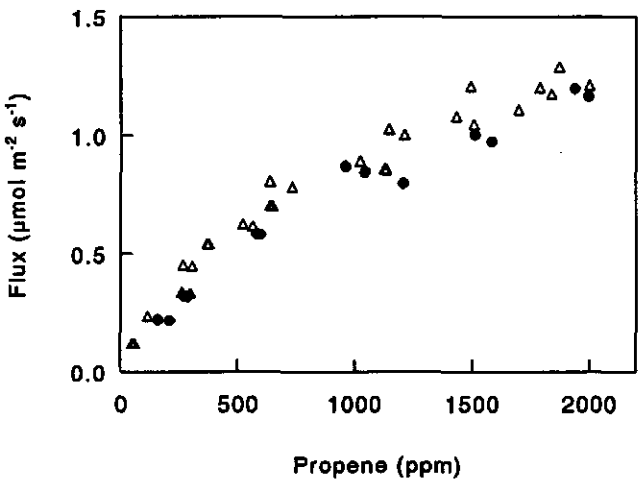

Figure 2. Flux of propene into the biofilm as a function of the propene concentration in the gas phase for strains Py1 (Δ) and Py2 (●), grown with ≈ 2300 ppm of propene in the gas phase. Fluxes were calculated from steady-state measurements.

Biofilms of Py1 and Py2 were visible after 14 and 5 days, respectively. Then the supply of propene into the gas loop was changed daily. Each resulting steady-state propene concentration was measured after 16 to 24 hours and from the combination of supply rate and measured concentration the consumption rate was calculated. In Figure 2 the flux of propene is shown versus the gaseous concentration. The performances of both strains were comparable, although the start-up period for *Mycobacterium* Py1 lasted considerably longer than for *Xanthobacter* Py2.

Kinetics of propene degradation by suspended cells

According to Michaelis-Menten kinetics the consumption rate at low concentrations is limited by the substrate concentration and in the lower concentration range the affinity of the reaction is important. For both strains we determined the kinetics of the first step in the degradation of propene with non-growing, propene-grown cells assuming that this reaction can be described by Michaelis-Menten kinetics. The consumption rates of *Mycobacterium* Py1 are shown in both a Lineweaver-Burk plot and an Eadie-Hofstee plot. Both graphic methods result in the same values for the half-rate constant (K_m), and the maximum consumption rate (V_{max}). Table 2 summarizes the resulting parameters.

Table 2. Kinetic parameters of propene degradation by two strains.

strain	max. reaction rate (V_{max}) [nmol min^{-1} mg^{-1}]	Michaelis-Menten constant (K_m) [ppm in air]	max. growth rate (μ) [h^{-1}]	reference
Py1	15	18	0.035	this work
Py2	75	160	0.14	15

Assuming that the first step in the biodegradation of propene is growth-rate limiting, the maximum consumption rates can be compared to the maximum growth rates. The biomass yield of *Xanthobacter* Py2 was previously shown to be 0.44 gC_{eq} biomass per gC_{eq} propene (15). With an assumption for the carbon content of the biomass (45%) the maximum consumption rates can be calculated from the μ_{max}-values. This results in a V_{max} of 66 nmol min^{-1} mg^{-1} for Py2 and 17 nmol min^{-1} mg^{-1} for Py1. These values are quite close to V_{max}-values of 75 and 15 nmol min^{-1} mg^{-1} measured with non-growing cells.

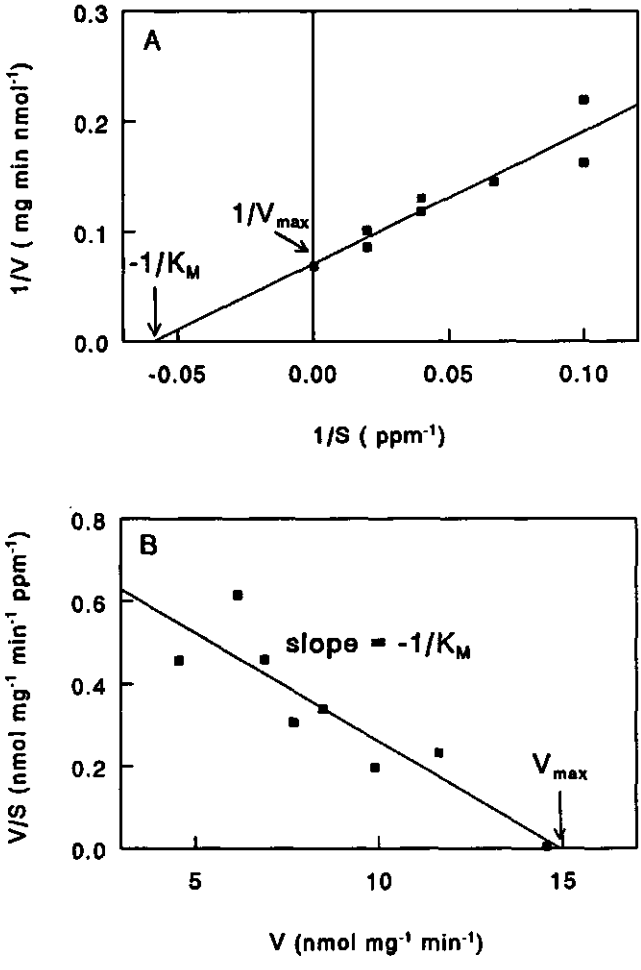

Figure 3. Kinetics of propene degradation by washed, non-growing *Mycobacterium* Py1 cells plotted as Lineweaver-Burk plot (A) and Eadie-Hofstee plot (B). V = propene consumption rate and S = propene concentration in the gas phase.

Using Michaelis-Menten kinetics and the data in Table 2, the specific consumption rates of both strains were calculated over a wide range of propene concentrations (Figure 4). Although *Xanthobacter* Py2 has a considerably lower affinity for propene than *Mycobacterium* Py1, the specific consumption rates of Py2 are higher than for Py1.

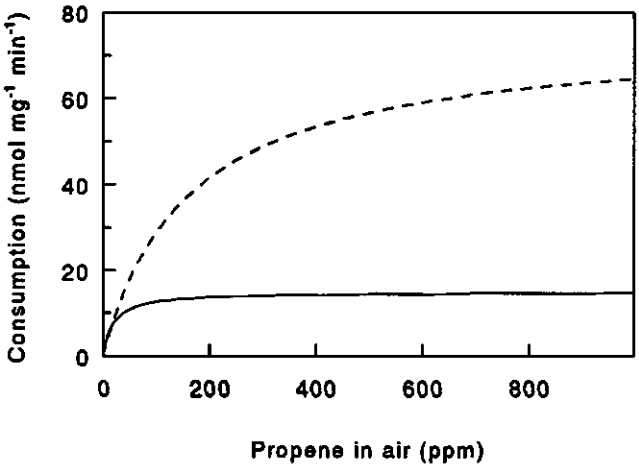

Figure 4. Propene consumption rates of *Mycobacterium* Py1 (——) and *Xanthobacter* Py2 (- - - -) as a function of the propene concentration in the gas phase. Data are calculated assuming Michaelis-Menten kinetics and using the parameters shown in Table 2.

Induction of the enzymes required for propene degradation

For continuous removal of propene from the gas phase not only the consumption rate is important, but it is also imperative that the biocatalyst is continuously regenerated. Consequently, permanent induction and production of the enzymes responsible for propene degradation are required. Propene is known to act as an inducer (6, 22) and we tested whether there is a minimal concentration of propene to trigger the production of the required enzymes.

Succinate-grown cells of both strains, that did not contain a detectable monooxygenase activity, were exposed to propene and the propene concentration was monitored in time (Figure 5). With *Xanthobacter* Py2 degradation of the propene started after 60 to 90 minutes, even at the lowest concentration tested, which was 5 ppm in the gas phase. With *Mycobacterium* Py1 induction was more difficult. In several experiments the induction period lasted more than 12 hours, or propene was not degraded at all for 7 days (data not shown). However, in a number of experiments in which mid-log phase cells were used, propene degradation started ≈ 3 hours after the addition of propene, even at 5 ppm. In the control bottles protein synthesis was inhibited by 50 μg ml^{-1} of chloramphenicol and the propene concentrations indeed did not decrease.

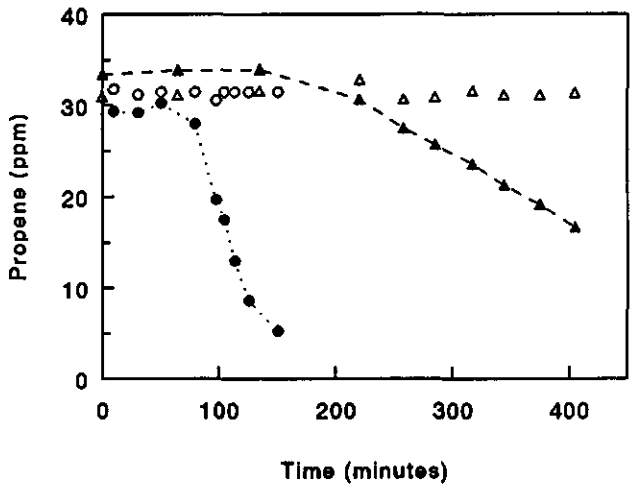

Figure 5. Induction of propene degradation in succinate-grown cells of *Xanthobacter* Py2 (●) and *Mycobacterium* Py1 (▲); Open symbols: corresponding controls amended with 50 μg ml^{-1} chloramphenicol.

Chloramphenicol did not inhibit monooxygenase activity already present in propene-grown cells (data not shown).

Concluding it can be said that a concentration as low as 5 ppm of propene in the gas phase is sufficient to induce the enzymes required for the degradation of propene, so continuous removal of propene down to the ppm-level seems feasible. It should, however, be noted that the cells used in these experiments were freshy harvested and may have contained considerable amounts of storage material. This is especially true for *Xanthobacter* Py2 which had formed slime during growth on succinate and might have used the slime as an additional source of carbon and energy during induction. Since the minimum amount of energy required for survival is not known, induction is no garuantee for long-term stability of the microorganisms at these low concentrations.

Selection of one strain for biofilm experiments at low concentrations

The kinetic parameters of propene consumption by *Mycobacterium* Py1 and *Xanthobacter* Py2 differ considerably and strain Py2 has the higher specific consumption rate (Figure 4). These kinetic parameters are not in agreement with the very similar performances of both strains in a biofilm reactor with a high concentration of propene (Figure 2). One possible explanation could be that the density of active cells in a biofilm is significantly higher for Py1 than it is for Py2.

Another important criterium for selection is the time required for biofilm start-up. At a high concentration of propene *Xanthobacter* Py2 formed a biofilm more rapidly than *Mycobacterium* Py1. Induction of the enzymes required for propene degradation was also easier with strain Py2. Hence, *Xanthobacter* Py2 was chosen for testing biofilm formation and propene removal at low concentrations in a membrane bioreactor.

Biofilm formation in a membrane bioreactor at low concentrations

On day 0 the reactor was inoculated with *Xanthobacter* Py2 cells. Figure 6a shows the flux of propene through the membrane and the supply into the gas loop (the theoretical maximum flux), both in μmol m^{-2} s^{-1}, for each of the 5 periods of this experiment.

During the first days of operation the propene flux through the membrane decreased, probably due to starvation and subsequent inactivation of microorganisms in the liquid phase. From day 10 to 20, however, the propene flux through the membrane increased gradually while the propene concentration in the circulating gas phase decreased from 350 to 200 ppm. In the same period the formation of a biofilm could be observed visually by the yellow colour of the *Xanthobacter* cells accumulating on the liquid side of the membrane.

While biofilm formation with ≈ 2300 ppm of propene in the gas phase was straightforward and took only 5 days, the formation of a biofilm at concentrations slightly higher than the K_m, required an extended start-up period and it should be noted that several attempts were not successful.

Variation of propene concentration

From day 27 on [period (2)] the supply of propene into the gas loop was changed once a day. The resulting steady-state consumption rates are shown as a function of the propene concentration in Figure 7. In addition to the steady-state measurements dynamic measurements were performed on days 22 and 26. Using this dynamic method the kinetics can be assessed within a few hours, whereas steady-state measurements require at least one day per data point.

In Figure 7 it can be seen that dynamic data and the steady-state data agree closely. On day 26 the flux into the biofilm was higher than on day 22. This is in agreement with the steady-state performance of the biofilm (Figure 6A), that indeed improved between day 22 and day 26. On day 26 apparently a larger amount of active biomass was present in the reactor than on day 22.

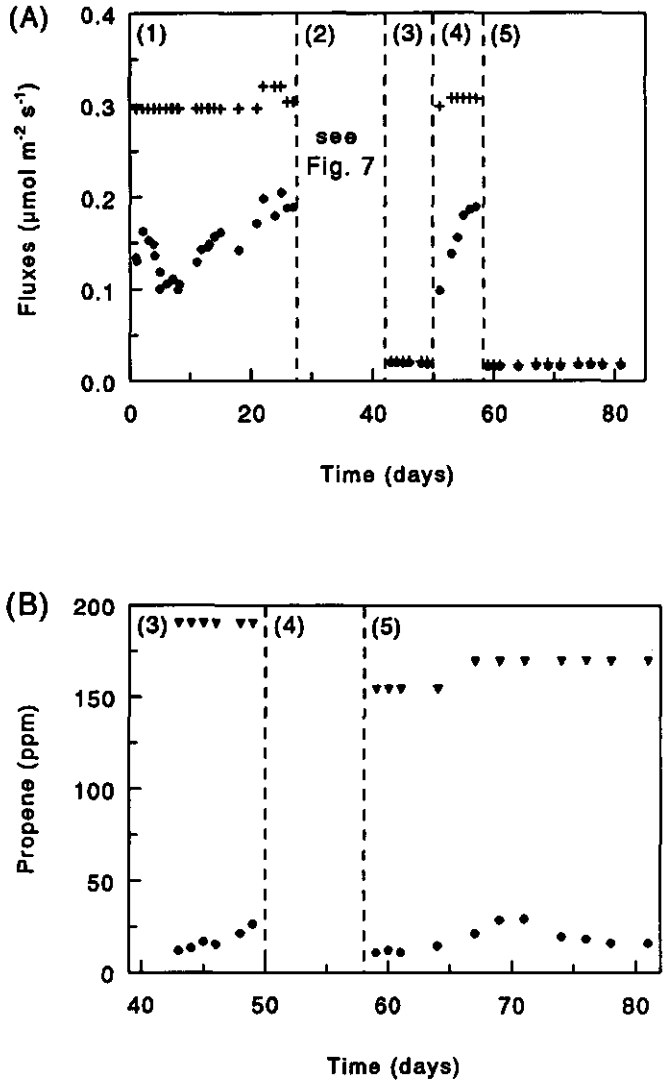

Figure 6. (A) Overview of a biofilm formation experiment. The five periods indicate inoculation and biofilm formation (1); variation in propene inflow (2); first period with very low concentration (3); biofilm recovery (4); second period with very low concentration (5). Symbols: flux of propene removed via the membrane (●) and theoretical maximal flux if the conversion were 100% (+); (B) Detailed view showing the concentration of propene in the gas supplied to the gas loop (▼) and the resulting concentration in the gas loop (●).

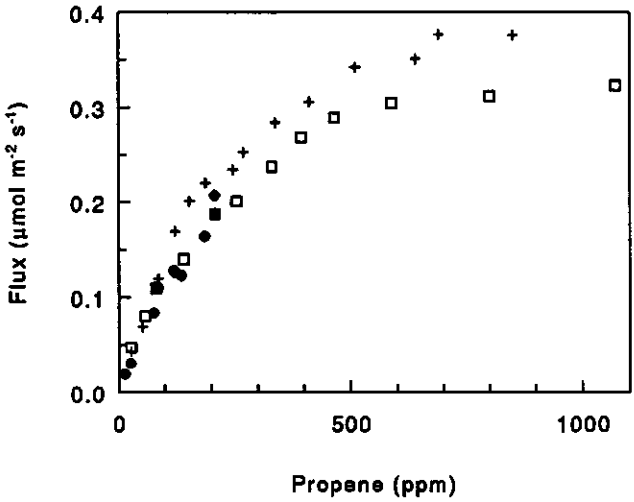

Figure 7. Fluxes of propene through the membrane as a function of concentration. Steady-state data (●) were measured between days 26 and 42. Dynamic measurements were performed on days 22 (□) and 26 (+).

First period at low concentrations and restoration

On day 42 [period (3)] the propene supply into the loop was reduced further to 0.02 µmol m^{-2} s^{-1}. The next day the propene concentration in the gas loop had dropped to 12 ppm, implicating that more than 90 % of the propene was being removed (Figure 6B). During the subsequent 6 days the propene concentration in the gas loop increased from 12 to 27 ppm, while the supply of propene was constant. The increasing concentration indicates loss of biological activity and therefore the biofilm was allowed to recover at a supply of 0.3 µmol m^{-2} s^{-1} during period (4). As can be seen in Figure 6a the flux into the biofilm indeed increased significantly during period (4). This increase occurred more rapidly than after inoculation, when 20 days were required to achieve the same activity.

Second period at a low concentration (< 30 ppm)

On day 58 the propene supply was reduced again and the stability of propene removal in the membrane bioreactor at low concentration was tested [period (5)]. On day 59 the resulting propene concentration was 11 ppm. After an increase to 29 ppm, probably due to an increase in the supply of propene, the concentration stabilized at a level of ≈ 20 ppm, which corresponds to an aqueous concentration of 0.09 µM. Apparently, *Xanthobacter* Py2 is able to remain viable at this concentration, which is well below a number of published threshold concentrations for carbon sources (3).

During period (5) the capacity of the biofilm was assessed dynamically on days 61 and 76 (Figure 8). On day 61, after 3 days at ≈ 10 ppm, the capacity of the biofilm was only slightly less than on day 22, when the biofilm had been operated with a concentration of ≈ 200 ppm. On day 76, however, the maximal propene flux into the biofilm was approximately halved compared to day 22. Apparently, the amount of active biomass in the biofilm adapts to the amount of propene available.

Thickness of the active biolayer

As can be seen in Figure 8 the observed flux into the biofilm on day 76 was maximally 0.16 μmol m^{-2} s^{-1}. Assuming the specific activity of *Xanthobacter* Py2 shown in Table 2, this maximum flux corresponds to the presence of 130 mg of biomass per m^2 of membrane. If 130 mg of biomass were distributed equally over 1 m^2 membrane, the biofilm would be considerably thinner than 1 μm, i.e. less than a monolayer of bacteria. Therefore, limitation of the flux by diffusion was neglected and the lower curve in Figure 8 was fitted with Michaelis-Menten kinetics using the half-rate constant from Table 2 (160 ppm).

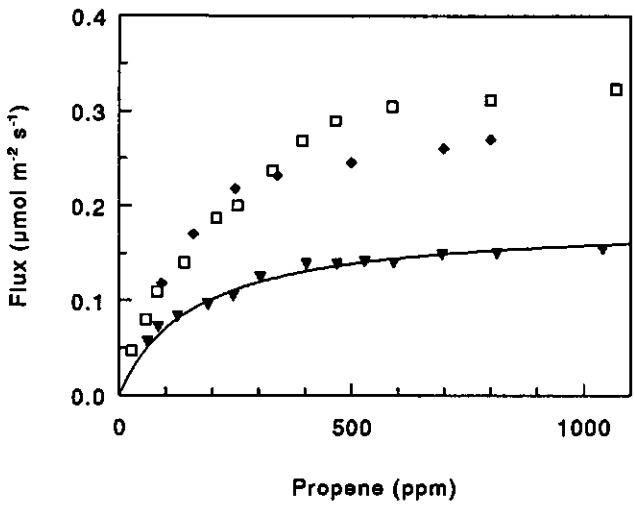

Figure 8. Effect of operating the membrane bioreactor at a low concentration of propene, on the removal capacity of a biofilm. Dynamic measurements were performed on day 22 (□), when the biofilm had been growing with ≈ 200 ppm propene and on days 61 (♦) and 76 (▼), when the concentration of propene had been less than 30 ppm for 2 resp. 17 days. The curve of day 76 was fitted using Michaelis-Menten kinetics and K_m = 160 ppm; V_{max} = 0.18 μmol m^{-2} s^{-1}.

The optimal fit of the maximal reaction rate was 0.18 μmol m^{-2} s^{-1} (r^2 = 0.98). According to this value and the concentration during steady-state operation (18 ppm), the flux into the biofilm on day 76 should have been 0.018 μmol m^{-2} s^{-1}. The actual steady-state flux into the biofilm on day 76 was 0.017 μmol m^{-2} s^{-1}, i.e. very close to the predicted value. Hence the propene consumption by a very thin (monolayer) biofilm growing on microporous membrane can be described quite accurately by Michaelis-Menten kinetics neglecting mass transfer limitation.

Comparison with reports from literature

Aliphatic hydrocarbons, such as methane and ethene, are known to be consumed in natural soil systems at concentrations around or below 1 ppm (2, 7). In bioreactors higher concentrations of aliphatics were continuously removed from air. In soil or compost biofilters 100-200 ppm of hexane (13), 10-250 ppm of n-butane (9) and 2,000 ppm of propane and butane (8) were partially removed from air. Trickle-bed reactors were also used for waste gas treatment. In such a bioreactor inert material is used as a carrier for biomass. Varying concentrations of isopentane were partially removed from air in a trickle-bed reactor (17) and air containing ≈ 100 ppm of ethene was treated using a packed bed of activated carbon (5).

Continuous removal of very low concentrations of aliphatics from air could up to now only be achieved in compost biofilters. Ethene was reduced from 2 ppm to less than 0.5 ppm by *Mycobacterium* E3 immobilized in compost (20). Immobilization of the same ethene-oxidizer on the inert substrata perlite and lava in the presence of 2 ppm of ethene, did not result in a stable system (19). Nutrients present in compost were presumably essential to maintain a viable population of ethene-oxidizers (7). Nevertheless, our experiments demonstrate that propene-degrading bacteria immobilized on inert membrane, were active for 25 days at concentrations ranging from 9 to 30 ppm.

CONCLUSIONS

The kinetic parameters of propene degradation by suspended cells of *Mycobacterium* Py1 and *Xanthobacter* Py2 differed considerably but the propene removal rates of biofilms of strain Py1 and strain Py2 were comparable at a high concentration of propene.

The formation of a biofilm of *Xanthobacter* Py2 took ≈ 20 days, when propene was supplied at a concentration in the same range as its K_m and when no additional source of carbon or energy was supplied to the biofilm. Propene removal was shown to be feasible at concentrations ranging from 10 to 1000 ppm in air.

Once the biofilm had been formed on the membrane the amount of active biomass adapted to the amount of propene available. After an increase in propene concentration from ≈30 ppm to ≈300 ppm the consumption rate adjusted within 5 days. When operated at a concentration of 9 - 30 ppm for several weeks, the total amount of active biomass decreased but the propene removal rate was stable.

To our knowledge this is the first report on a bioreactor with which a poorly soluble volatile can be removed from air for a prolonged period at very low concentrations (less than 30 ppm), without the supply of any other source of carbon or energy to the microbial population.

ACKNOWLEDGEMENTS

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CHAPTER 6

Continuous degradation of trichloroethene by *Xanthobacter* Py2 during growth on propene

Martine W. Reij, Jasper Kieboom, Jan A.M. de Bont, and Sybe Hartmans

Summary: Propene-grown *Xanthobacter* Py2 cells can degrade trichloroethene (TCE), but the transformation capacity was limited and depended on both the TCE concentration and the biomass concentration. Toxic metabolites presumably accumulate extracellularly, because the fermentation of glucose by yeast cells was inhibited by TCE degradation products formed by strain Py2. The affinity of the propene monooxygenase for TCE was low and allowed strain Py2 to grow on propene in the presence of TCE. During batch growth with propene and TCE, the TCE was not degraded before most of the propene had been consumed. Continuous degradation of TCE in a chemostat culture of *Xanthobacter* Py2 growing with propene, was observed with TCE concentrations up to 206 μM in the growth medium, without wash-out of the fermentor occurring. At this TCE concentration the specific degradation rate was 1.5 nmol per min per mg biomass. The total amount of TCE that could be degraded during simultaneous growth on propene depended on the TCE concentration and ranged from 0.03 to 0.34 g TCE per g biomass. The biomass yield on propene was not affected by the cometabolic degradation of TCE.

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INTRODUCTION

A variety of aerobic bacteria containing oxygenases have been reported to degrade the pollutant trichloroethylene (TCE) cometabolically when they are grown on aliphatic hydrocarbons (4, 14, 18), aromatic hydrocarbons (7) or ammonium (20). Ensign et al. (3) showed that the propene-oxidizer *Xanthobacter* Py2 is able to degrade TCE when it is grown on propene because of the presence of propene monooxygenase.

The toxicity of TCE is a major problem encountered in the aerobic degradation of this compound, and this toxicity is probably due to the formation of highly reactive intermediates. Methane oxidizers are harmed irreversibly, and the greater the amount of TCE that the cells have transformed, the greater the effect (1). The degradation of TCE by purified methane monooxygenase results in inactivation of the enzyme (8). Other enzymes have also been shown to be affected as a result of TCE degradation (3, 17). The nature of these inactivating effects is not clear, but it has been shown that TCE oxidation products cause alkylation of macromolecules (8, 17, 23). It has been shown that in *Nitrosomonas europaea* de novo protein synthesis is required to regain oxidation activity (20).

Because of the inactivation effects caused by TCE degradation, the transformation capacity of cells is limited (1, 11, 18) and processes for continuous removal of TCE consequently should involve regeneration of inactivated biomass. Continuous aerobic degradation of TCE in a bioreactor or field situation has been observed with methane-oxidizers (5, 15, 21) and microorganisms growing with aromatic substrates (7, 11, 13).

In this study *Xanthobacter* Py2 was chosen to study the continuous degradation of TCE to allow comparisons with data already reported for methane- and aromatic hydrocarbon-degrading bacteria. Since the TCE transformation capacity of cells is limited, synthesis of the monooxygenase is required for continuous TCE removal. Unfortunately, propene, which induces the alkene monooxygenase, also inhibits the oxidation of chlorinated alkenes (3).

The same problem has been encountered with TCE degraders growing on methane (5), phenol (11), propane (14) and toluene (13). In all of these cases the presence of the growth substrate resulted in restoration of monooxygenase activity, but inhibited TCE degradation. To overcome the problem of inhibition of TCE degradation by the growth substrate, workers have designed two-stage bioreactors (6, 15). In these types of reactors cells are supplied with growth substrate in the first stage and are

then transported to the second stage, where TCE is degraded. Other workers have studied alternating the supply of growth substrate and TCE (19). Nevertheless, under controlled conditions simultaneous growth and TCE degradation in only one reactor are also possible (5, 13, 16).

We used the continuous culture technique to characterize the kinetics and physiology of simultaneous propene degradation and TCE degradation in *Xanthobacter* Py2 cells. In this paper we first describe the transformation capacity and the kinetics of TCE oxidation, and then focus on TCE degradation during growth on propene in batch and continuous cultures.

MATERIALS AND METHODS

Organisms and culture conditions.
Xanthobacter Py2 was isolated with propene (22), and was grown continuously in a 1.5 l fermenter with mineral salts medium (9) at a dilution rate of 0.03 hr^{-1}; 1.25 % (vol/vol) propene in air was supplied at a rate of 100 ml min^{-1}. The impeller speed was 550 min^{-1}, the temperature was 30°C and the pH was kept constant at 7.0 by titration with 2 M NaOH. Cells were harvested by centrifugation, washed with washing buffer (50 mM potassium phosphate buffer, pH 7.0), and resuspended in the same buffer.

For batch growth in the presence of TCE (see Fig. 3), *Xanthobacter* Py2 pre-grown with propene, was inoculated into 10-ml portions of mineral salts medium in 250-ml serum flasks sealed with Teflon-lined Mininert septa (Alltech, Deerfield, Ill.). Propene was added in the gas phase to a final concentration of 1.1%. TCE was added as a saturated solution in mineral salts medium to a final concentration of 0, 36, 74, and 250 µM in the water phase. The bottles were incubated in a water bath at 30°C and the concentrations of CO_2, propene, and TCE were monitored by gas chromatography during the experiment.

For continuous growth with propene in the presence of TCE, *Xanthobacter* Py2 was grown in a stirred vessel as described above at a dilution rate of 0.034 (± 0.003) hr^{-1}. Propene was mixed with air and a third airflow, which was saturated with TCE in a saturation column at room temperature. All gas flows were controlled by thermal mass flow controllers (Brooks Instrument B.V., Veenendaal, The Netherlands). The propene concentration was 1.25 % and the total flow was 106 ml min^{-1}. In- and outgoing concentrations of TCE, propene, and CO_2 were determined daily by sampling two disconnectable 0.75-liter glass containers placed in the in- and outgoing gas streams. The amounts of propene and TCE lost abiotically were less than 3%. After each change in the TCE loading rate, the reactor was operated for 5 days (corresponding to four changes of volume) before steady-state was confirmed as follows: the in- and outgoing concentrations and the optical density at 660 nm were constant for at least another 2 days.

Saccharomyces cerevisiae (CBS 1394) was grown in 0.5-liter Erlenmeyer flasks that contained 0.3 liter medium supplemented with 10 g of yeast extract per liter and 10 g of glucose per liter and were incubated at 30 °C in a rotary shaker. Cells were harvested by centrifugation, washed with 50 mM potassium phosphate buffer, and resuspended in the same buffer. Suspensions were kept on ice and used within a few hours after they were harvested.

TCE and propene degradation assays.

The transformation capacities of washed propene-grown cells were determined in 250-ml serum flasks sealed with Teflon-lined Mininert septa. TCE was added as a saturated solution (8.3 mM) in 50 mM potassium phosphate buffer at room temperature, to final concentrations of 130, 260, and 390 µM. Washed *Xanthobacter* Py2 cells were added at a final concentration of 1.8, 3.6, or 5.5 mg (dry weight) per ml, and the final volume was 10 ml. The flasks were incubated in a water bath at 30 °C, and the concentration of TCE was followed for 20 hours by gas chromatography. After 20 h the concentration of the accumulated Cl^--ions in the medium was determined.

Kinetic parameters of propene degradation were determined at 30°C in rubber-sealed 75-ml serum vials containing 5 ml of 50 mM potassium phosphate buffer (pH 7.0) and 0.15 to 0.37 mg of cells. To eliminate the possibility that the reaction was limited by diffusion, two cell densities were incubated with vigorous shaking. Since the specific degradation rates were found to be the same for the two cell densities, we concluded that the degradation rate was not limited by diffusion.

The TCE degradation assays were performed with 0.25 mg of cells at 30°C in 250-ml serum flasks sealed with Teflon-lined Mininert septa and contained 25 ml of 50-mM potassium phosphate buffer. The initial rates of TCE degradation were determined for the first 15 minutes.

Toxicity of extracellular metabolites to *Saccharomyces cerevisiae*.

A 1.7-mg portion of washed *S. cerevisiae* cells was incubated aerobically in a water bath at 30°C with 10 or 20 mg of *Xanthobacter* Py2 cells and 64 µM of TCE in 250-ml serum flasks that were sealed with Teflon-lined Mininert valves and contained a total liquid volume of 10 ml. After 2.5 hours the Mininert valves were exchanged for rubber seals, and the flasks were flushed with nitrogen for 30 minutes to remove oxygen, CO_2, and residual TCE. Then glucose was added to a concentration of 5 g l^{-1}, and the production of CO_2 was monitored.

Analytical methods

The amounts of TCE, propene, and carbon dioxide were determined by gas chromatography of 100-µl headspace samples. TCE was analyzed in triplicate with a Packard model 437 gas chromatograph equipped with a 20% Tween column (Chromosorb W AW 80-100; Chrompack, Middelburg, The Netherlands) and a flame-ionization detector. The column temperature was 110 °C. The concentration of TCE was expressed as the actual

micromolar concentration in the liquid phase which the cells experienced. The total amount of TCE was calculated by adding the amounts present in liquid and gas phases and using a TCE gas/water partition coefficient of 0.5 (2).

Propene was analyzed in triplicate with a Packard model 430 gas chromatograph fitted with a Porapak R column (100-120 mesh, 110 [ca. 279 cm] × 1/8 in. [ca. 3.2 mm] [inside diameter]) and a flame ionization detector. The oven temperature was 180 °C, and the carrier gas was N_2. CO_2 was analyzed in duplicate with a Packard model 427 gas chromatograph fitted with a HayeSep Q column and a thermal conductivity detector at 140 °C. The column temperature was maintained at 110 °C, and helium was used as the carrier gas. The standard deviations of TCE, propene, and CO_2 measurements were 3, 2, and 1 %, respectively. Dry weights were determined by centrifuging 50-ml samples, resuspending the resulting preparations in demineralized water, and drying them at 108 °C.

Chloride ion concentrations were determined in triplicate by using cell-free supernatants and a microchloro counter (Marius, Utrecht, The Netherlands), which titrated the chloride ions with Ag^+ released electrochemically from a silver electrode.

Chemicals

TCE (99.5% pure) was purchased from E. Merck, Darmstadt, Germany. Propene (99.995% pure) and N_2 (99.99% pure) were purchased from Hoekloos, Schiedam, The Netherlands. All other chemicals were reagent grade.

RESULTS

Transformation capacity of washed cells

Before we studied TCE degradation by a continuous culture of *Xanthobacter* Py2, we determined the transformation capacity of washed propene-grown cells (i.e., the total amount of TCE that could be degraded by these cells in the absence of other substrates). The effects of both cell density and TCE concentration on transformation capacity were studied. Cells were incubated with TCE for 20 h, after which no residual monooxygenase activity was detected. Table 1 shows the amounts of TCE eliminated per gram of biomass as determined by gas chromatography as well as by titration of the accumulated Cl^--ions. Most of the chlorine atoms in TCE eventually accumulated in the medium as Cl^--ions. Our results show that the specific transformation capacity of the culture decreased with cell density, suggesting that cells produced toxic degradation products which accumulated extracellularly.

Effect of TCE degradation products on glucose fermentation by *S. cerevisiae*

To test the hypothesis that toxic intermediates appeared extracellularly, the following experiment was performed. *S. cerevisiae* was incubated aerobically for 2.5 h in potassium phosphate buffer in the presence of *Xanthobacter* Py2 and TCE.Then the incubation system was flushed with N_2, glucose was added and the CO_2- production by *S. cerevisiae* during anaerobic incubation was measured. In several experiments (data not shown) the fermentation rate of *S. cerevisiae* was reduced by 20 to 28 % by the preceding incubation if TCE and *Xanthobacter* Py2 had been present simultaneously.

The results of one of these experiments are shown in Figure 1. Preincubation with either TCE or strain Py2 did not reduce the fermentation rate. Anaerobically, *Xanthobacter* Py2 did not produce any CO_2.

Table 1. Effects of cell density and TCE concentration on the amount of TCE degraded by washed propene-grown *Xanthobacter* cells[a]

Amount of biomass (mg ml^{-1})	Amount of TCE degraded at an initial TCE concentration of:					
	130 µM		260 µM		390 µM	
	TCE assay	Cl^- assay	TCE assay	Cl^- assay	TCE assay	Cl^- assay
1.8	43.6 ± 6.5	30.6 ± 1.2	55.6 ± 11.5	37.1 ± 1.3	58.2 ± 16.4	42.3 ± 1.1
3.6	43.1 ± 2.3	25.4 ± 0.3	47.3 ± 6.4	34.4 ± 0.1	46.9 ± 10.0	36.4 ± 0.2
5.5	35.0 ± 1.8	27.4 ± 0.5	39.4 ± 3.7	29.6 ± 0.3	43.3 ± 6.7	32.7 ± 0.6

[a] TCE removal was determined after 20 hours of incubation. Values are expressed in milligrams of TCE oxidized per gram of biomass and were determined by the removal of TCE and by the accumulation of Cl^--ions. We assumed that three chlorine atoms represented one molecule of TCE degraded.

Kinetics of the alkene monooxygenase

Using propyne as a specific inhibitor of the monooxygenase, Ensign et al. (3) have shown that the propene monooxygenase is required for TCE degradation. Therefore, we studied the kinetics of the degradation of both TCE and propene, which is the inducer of the propene monooxygenase. First, the initial degradation rates of both substrates by washed cells were studied as a function of the concentrations of the substrates. Reliable measurements of TCE degradation rates were possible only at concentrations lower than the resulting K_m (116 µM). The initial TCE degradation rates were determined during the first 15 min. Figure 2 shows that Xanthobacter Py2 had a higher affinity and a higher maximal degradation rate for propene than for TCE.

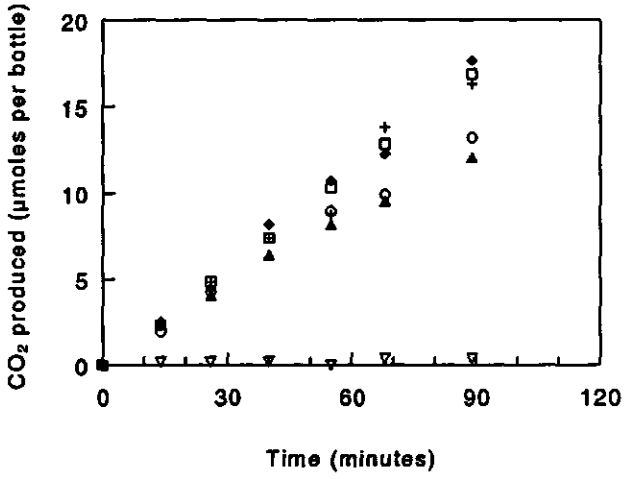

Figure 1. Effect of preceding aerobic incubation with TCE and strain Py2 on anaerobic CO_2 production from glucose by *S. cerevisiae*. The yeast cells were pre-incubated aerobically in the presence of 64 µM TCE (♦), 10 mg of strain Py2 (+), without additions (□), 10 mg of strain Py2 and 64 µM TCE (▲), 20 mg of strain Py2 and 64 µM TCE (O). ▽, control containing only 10 mg of strain Py2.

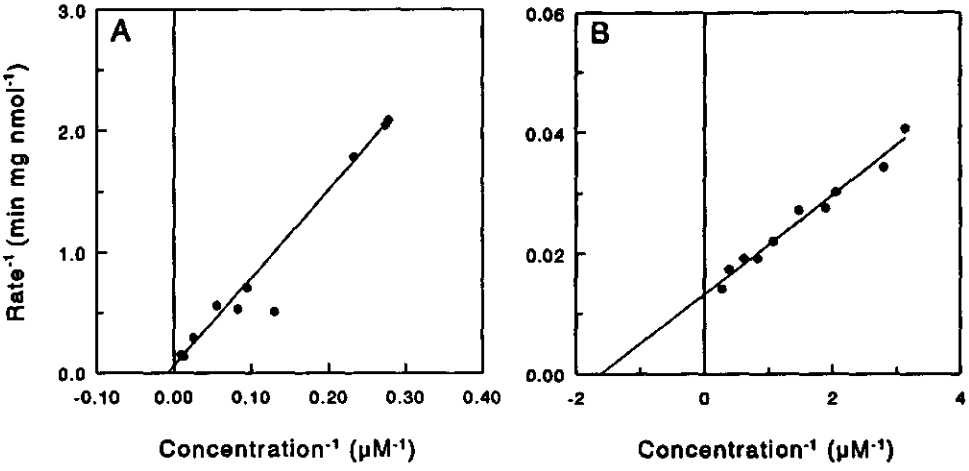

Figure 2. Lineweaver-Burk plots of TCE degradation (A) and propene degradation (B) by *Xanthobacter* Py2. Concentrations are expressed in micromoles per liter liquid phase. The parameters resulting from the linear least-square fit (the outlying data point in A was omitted from the fit) are as follows: for TCE, V_{max} = 16 nmol min^{-1} mg^{-1} and K_m = 116 µM; and for propene, V_{max} = 75 nmol min^{-1} mg^{-1} and K_m = 0.62 µM.

Subsequently, the kinetics of simultaneous TCE degradation and propene degradation were studied. High concentrations of propene inhibited the degradation of TCE (data not shown but this phenomenon is shown in Figures 3B to D). To demonstrate that propene degradation was inhibited by the presence of TCE, high concentrations of TCE (concentrations greater than the K_m) were required, since the affinity of the monooxygenase for TCE is much lower than its affinity for propene. In the presence of TCE concentrations higher than the K_m for TCE, the propene oxidation rate decreased rapidly during the experiment.

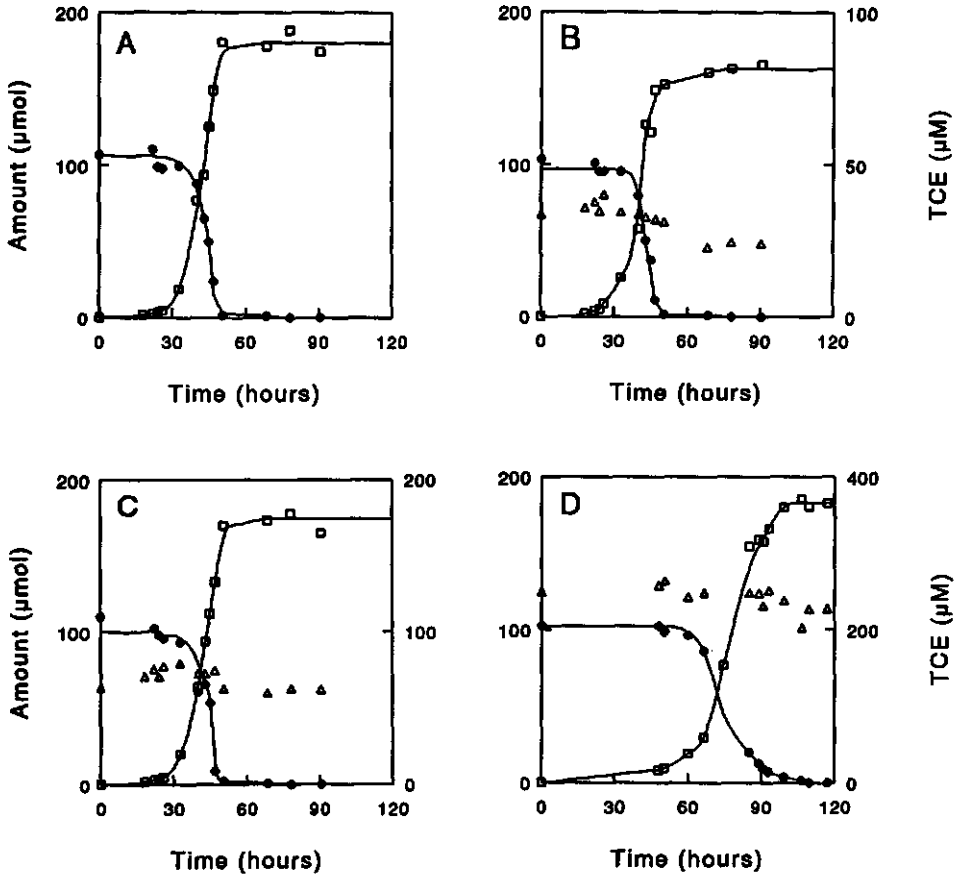

Figure 3. Effect of TCE on the growth of *Xanthobacter* Py2 on propene. The initial TCE concentrations were 0 µM (A), 34 µM (B), 76 µM (C), and 250 µM (D). The *y* axes on the left indicate the propene (●) and CO_2 (□) concentrations (in µmoles per flask); The *y* axes on the right indicate the TCE concentrations (△) (in µmoles per liter of liquid).

This was probably due to irreversible loss of enzyme activity because of simultaneous TCE oxidation. Therefore, the inhibition constants (K_i) of TCE and propene could not be determined.

Batch growth on propene in the presence of TCE

Before we studied continuous degradation of TCE during growth on propene in a continuous culture, we studied batch growth of *Xanthobacter* Py2 in the presence of TCE. Figures 3 shows the growth curves of *Xanthobacter* Py2 when the initial concentrations of TCE in the water phase were 0, 36, 74, and 250 μM. The maximal growth rate (as determined from the CO_2 formation curve) in the presence of 0 to 74 μM TCE was 0.14 (± 0.02) hr^{-1}. In the presence of 250 μM TCE the growth rate decreased to 0.08 hr^{-1}. Figure 3 also shows that TCE degradation did not start before most of the propene had been consumed. This is consistent with the affinity of washed cells for the two substrates (Figure 2), assuming that both substrates are oxidized by the same enzyme.

Continuous growth on propene in the presence of TCE

Xanthobacter Py2 was grown in a chemostat culture at a dilution rate of 0.034 (± 0.003) hr^{-1}, which is well below the maximal growth rate observed in the presence of 250 μM of TCE (Figure 3D). For each TCE loading rate the reactor was operated for four volume changes, and steady-state was confirmed by the in- and outgoing concentrations of TCE, and by the fact that the optical density at 660 nm was constant for at least another 2 days. Figure 4 shows that *Xanthobacter* Py2 grew propene-limited in the presence of TCE.

The amount of TCE degraded increased as the TCE concentration increased, although the level of TCE conversion decreased (Figure 4A). The highest TCE load tested (461 μmol l^{-1} hr^{-1}) resulted in a steady-state concentration in the liquid phase of 206 μM and a volumetric TCE removal rate of 54 μmol l^{-1} hr^{-1}. Figure 5 shows the amount of TCE removed per gram of biomass as a function of the TCE concentration in the liquid phase.

The rate of carbon recovery was between 85 and 112 % (Fig. 4B), indicating that the overall variability of the data was limited. The yields of biomass and CO_2 on propene were constant (Fig. 4B) irrespective of the TCE concentration in the liquid. The constant biomass yield suggests that TCE degradation did not cause a significant increase in the maintenance requirements due to cell damage or loss of reducing equivalents. An interruption in the propene supply for several hours at the highest TCE concentration tested, caused a wash-out of the fermentor.

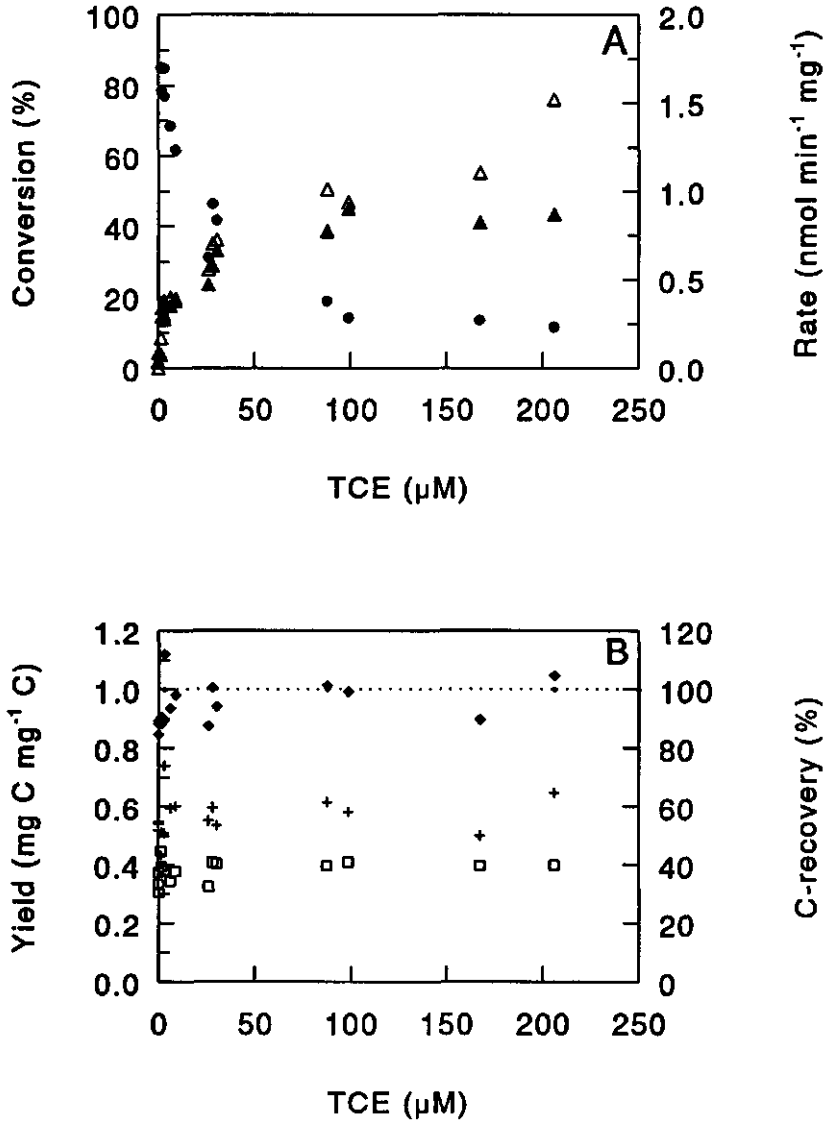

Figure 4. TCE degradation by strain Py2 in continuous cultures during growth on propene. The TCE load was varied between 15 and 461 µmol liter^{-1} hr^{-1} and resulted in steady-state concentrations in the liquid phase (indicated on the x axes). (A) Symbols: ●, level of TCE conversion; △, TCE removal rate as determined by gas chromatography; ▲, removal rate as determined by the accumulation of Cl^-. (B) Yields (in milligrams of C/milligrams of C) of biomass (□) and CO_2 produced (+) and rates of recovery (♦). For biomass a C-content of 0.45 g carbon per g biomass was assumed.

DISCUSSION

Xanthobacter Py2 cells were able to degrade a limited amount of TCE, and this amount varied with the cell density (Table 1). In the presence of the highest biomass concentration (5.5 mg of biomass per ml) and lowest TCE concentration (130 μM) tested, 87 % of the TCE was degraded. The lower initial TCE concentrations used in this experiment are in the range of the K_m value for TCE (116 μM). Therefore, the lower specific transformation capacity at a high cell density could have been due to substrate limitation. At the highest TCE concentration tested (390 μM), however, a maximum of 30 % of the compound was converted. Therefore, in this case the decrease in transformation capacity with increasing cell density could not have been due to kinetic effects. We speculate that toxic metabolites, (e.g., carbon monoxide [10], hydrolysis products of the TCE epoxide or chloral [8]) accumulate in the medium to a greater extent at higher cell densities, resulting in stronger inhibition. Alvarez-Cohen and McCarty (1), however, found the opposite effect of cell density on transformation capacity. In mixed methanotrophic cultures the transformation capacity was slightly higher at higher cell density. The values reported by these authors (0.025 to 0.033 g of TCE per g of biomass) (1) are similar to the values which we found for *X.* Py2. With phenol-oxidizing microorganisms Hopkins et al. (11) observed a much higher transformation capacity (0.24 g TCE per g of cells).

Our hypothesis that toxic metabolites appear extracellularly, was supported by the observed inhibition of yeast cells (Figure 1) incubated with *Xanthobacter* Py2 cells degrading TCE. Incubation with either TCE or strain Py2 had no effect on the fermentation rate of the yeast, clearly demonstrating that inhibitors were formed during incubation with both TCE and strain Py2. The nature of the inhibitory products formed from TCE by strain Py2 is not known. Because of its volatile nature carbon monoxide is unlikely to play a role, since the bottles were flushed with N_2 before glucose was added. Diffusible hydrolysis products of the TCE epoxide or chloral, however, might be responsible for the observed inhibition. Such hydrolysis products have been found to inactivate the purified methane monooxygenase by covalent modification (8). On the other hand, compounds produced by *Xanthobacter* itself as a stress response to TCE could also be responsible for the inhibition of the yeast. Irrespective of the nature of these inhibitors, degradation of TCE presumably results in the extracellular appearance of toxic metabolites. This observation has great impact on the TCE removal process and implies that biomass and liquid from a reactor in which TCE is degraded should be handled with care.

The Michaelis-Menten half-saturation constant (K_m) for TCE was found to be 116 µM, which is considerably higher than the values reported for *Pseudomonas cepacia* G4, 3 µM (7) and 6 µM (13). Our half-saturation constant value was, however, in the same range as the values found for the methane oxidizers *M. trichosporium* OB3b (145 µM [17] and 126 µM [12]) and *M. methanica* 68-1 (225 µM [12]).

Propene and TCE, which are probably substrates for the same enzyme (3), influence the rate of degradation of the other. Propene oxidation rates decreased rapidly in the presence of excess TCE. On the other hand, a high concentration of propene prevented TCE degradation (Figures 3B to D). In this context it should be noted that the specificity constant (maximum rate of metabolism [V_{max}]/K_m) for propene is approximately 900 times greater than the specifity constant for TCE. The low specificity constant of the enzyme for TCE thus allows batch growth on propene in the presence of 250 µM TCE (Fig. 3D). During batch growth TCE is not degraded until most of the propene has been consumed. A similar pattern of subsequent substrate degradation was found for the isoprene-utilizing *Rhodococcus erythropolis* JE77 (4).

The mutual influence of growth substrate utilization and TCE degradation has also been observed with various other organisms (6, 11-14, 16). Folsom et al. (7) suspected that the influence of phenol on TCE oxidation and vice-versa in *P. cepacia* G4 can be explained by a competitive mechanism. Landa et al. (13) have actually report K_i values for competitive inhibition by toluene during TCE oxidation and vice versa with the same organism; the reported values (K_i for toluene, 30 µM; K_i for TCE, 5 µM) are very close to the independently determined K_m values (25 µM and 6 µM, respectively), suggesting that competitive inhibition occurs, but no Lineweaver-Burk plots are given by these authors. For *Xanthobacter* Py2 the type of inhibition could not be established because of the quick and irreversible loss of enzyme activity in the presence of high concentrations of TCE.

The specific TCE degradation rates by cells during growth on propene in the fermentor (Fig. 4) were significantly lower than the initial rates (e.g., at 100 µM 7.4 nmol min^{-1} mg^{-1}) determined in the absence of propene. The lower rates in the fermentor were probably due to competition between propene and TCE for the monooxygenase and possibly also because of lower levels of active monooxygenase in cells growing in the presence of TCE.

Although the specific rates of TCE degradation rates in the chemostat were relatively low, the transformation capacity of the cells (0.34 g g^{-1}) (Figure 5) was six- to seven-fold higher than the amount of TCE degraded by washed cells in the

absence of propene (Table 1) at a similar concentration of TCE. The higher transformation capacity in the presence of propene may have been due to replenishment of reducing equivalents by propene oxidation. In phenol-oxidizing microorganisms the TCE degradation capacity was improved by 102 % in the presence of an external source of reducing equivalents (1). With a methanotrophic culture the energy- generating substrate formate was found to increase the transformation capacity by 58 %, indicating that depletion of stored energy reserves by TCE oxidation was a factor which determined the transformation rate and capacity (11). However, apart from the replenishment of reducing equivalents by propene oxidation, de-novo synthesis of the propene monooxygenase in the presence of the inducer propene is probably the main reason for the increased transformation capacity.

Other workers (Table 2) have reported similar values for the transformation capacity of toluene and phenol oxidizers (11, 13), whereas lower values have been reported for methane oxidizers (16). Table 2 also shows the amount of growth substrate required for degradation of TCE. The values in Table 2 are very difficult to compare since they depend not only on the TCE concentration but also on whether the growth substrate and TCE were supplied alternately. In general, substantial amounts of volatile growth substrate(s) are required for cometabolic degradation of TCE, which results in high costs and contamination of air or water with the residual growth substrate(s).

Table 2. Amounts of growth substrate required per gram of TCE degraded and transformation capacity of the biomasses during continuous degradation.

Preparation	Growth substrate(s)	Amount of substrate (g of substrate consumed per g of TCE degraded)	Transformation (g of TCE per g of biomass)	Ref.
mixed culture	methane	23-1,200	-	5
mixed culture	methane	77 [a]	0.036 [a]	1
mixed culture	methane + propane	11-30	0.015-0.08 [b]	19
*M. trichosp.*OB3b	methane	320-1,200	1.7×10^{-4}-6.8×10^{-3}	16
mixed culture	phenol	9 [a]	0.24 [a]	11
P. cepacia G4	toluene	14-71	0.027-0.152	13
Xanthobacter Py2	propene	4-23	0.03-0.34	this work

[a] Degradation capacity determined in the absence of energy-generating substrates.

[b] Per day.

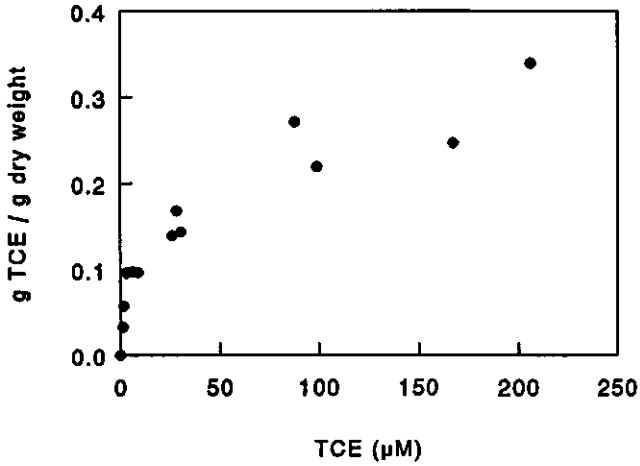

Figure 5. Amount of TCE removed per gram of biomass during continuous growth of strain Py2 on propene. Data were obtained from Fig. 4A.

Although a continuous culture of *X.* Py2 can grow in the presence of a wide range of TCE concentrations, it is susceptible to interruptions in the propene supply at a high concentration of TCE, which causes a wash-out of the fermentor. The cells are apparently not able to recover from exposure to TCE in the absence of propene. Thus, although simultaneous oxidation of propene during TCE degradation results in lower rates of TCE degradation, it also prevents total inactivation of the biocatalyst.

The biomass yield of *X.* Py2 on propene was not affected by the simultaneous TCE degradation, even when the concentration of TCE in the liquid was 206 µM TCE (Figure 4B). With the toluene-oxidizing *P. cepacia* G4 Landa et al. (13) also found a constant biomass yield over a wide range of TCE concentrations up to 14 µM, but at 80 µM the yield was lower. On the basis of the energy required for the initial step in the oxidation of TCE (1 NADH), a detectable decrease in the yield can hardly be expected when the propene flux (at least 15 nmol min^{-1} mg^{-1}) is compared with the TCE flux (1.5 nmol min^{-1} mg^{-1} at most). However, we anticipated that an increase in the monooxygenase turnover rate due to inactivation by TCE, would increase the apparent maintenance requirements and hence decrease the yield on propene. However, it is possible that the inactivation of the cells by TCE degradation was compensated for with energy gained from the oxidation of TCE degradation products.

In conclusion, our results clearly demonstrate that *Xanthobacter* Py2 cells growing on propene, can be used to continuously degrade TCE over a wide range of concentrations. However, a disadvantage of this organism is its low affinity for TCE, which results in rather low specific degradation rates at low TCE concentrations.

ACKNOWLEDGEMENTS

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CHAPTER 7

General discussion

To conclude this thesis I will evaluate the potential of membrane bioreactors for the removal of poorly water-soluble pollutants from air. Since stable operation is essential for full-scale application, I will subsequently focus on the long-term stability of biofilm activity in a membrane bioreactor and methods to assess the microbial activity. Finally, I will describe the possible applications of membrane bioreactors in biofilm research and for the removal of highly chlorinated hydrocarbons, such as trichloroethene, from contaminated air or water.

Poorly water-soluble pollutants

Pollutants with a low water-solubility are difficult to remove from air with conventional gas-liquid contactors, due to mass transfer limitations in the liquid phase. New techniques for the removal of these pollutants are required (12). In membrane bioreactors a large gas-liquid interface can be created and the mass transfer resistance of the membrane for this type of pollutants can be neglected (Chapter 3). Therefore, the treatment of waste gases containing poorly water-soluble pollutants can be considered to be the most promising application of membrane bioreactors.

In addition to the membrane bioreactor, other reactor types were found to be successful in the removal of poorly soluble pollutants from air during prolonged experiments. Morgenroth et al. (17) showed that hexane, which has an extremely low solubility, can be removed from air using conventional compost biofilters. They concluded that the nutrient supply and the moisture control are critical. De Heijder et al. (5) immobilized ethene-degrading bacteria on activated carbon and varied

the humidity of the gas stream. The ethene removal rate was optimal at relatively dry conditions. The latter studies indicate that stringent humidity control is a promising technique in biological waste gas treatment. Cox et al. (3) tested the use of styrene- degrading fungi in bifilters. Fungi are more resistant to acidic and dry conditions than bacteria. As a consequence, control of a fungal biofilter is not as critical as it is with bacteria and fungal biofilters may have a wider applicability.

Toxic end-products of biological degradation, however, can hardly be removed from biofilters and dry biobeds. If biodegradation of a poorly soluble pollutant results in acidification, the membrane bioreactor is the only configuration which allows removal of acidic products via the liquid phase.

Stability of the biological activity

In this thesis it was shown that propene removal is feasible over a wide range of concentrations (10-6000 ppm) (Chapters 4 and 5). The start-up of the membrane bioreactor was shown to be difficult when only very low concentrations of propene were supplied (Chapter 5) probably due to starvation and subsequent inactivation of the microorganisms that had been inoculated in the liquid phase.

For the degradation of very low concentrations of, particularly poorly soluble, pollutants, a compost biofilter may be advantageous, since microorganisms benefit from nutrients present in the compost. A possible option to improve the removal of low concentrations in a membrane bioreactor, is the supply of additional growth substrates. These substrates should sustain the desired population, without interfering with the degradation of the pollutant and without causing excessive growth.

In hollow-fibre bioreactors a generous supply of ammonium served as an additional source of energy and brought about a population of nitrifiers. The nitrification process resulted in acidification of the liquid and a decrease in oxygen tension (Chapter 4). The propene degradation was thus hampered instead of stimulated by the use of ammonium as additional substrate.

In addition to nitrification, another problem was observed during the long-term operation of hollow-fibre bioreactors at a high concentration of propene. Biomass accumulated in the reactor and eventually blocked most of the fibres (Chapter 4). This phenomenon is called clogging and was also observed with other membrane bioreactors studied for gas and water treatment (9,19), but also in trickle-bed-reactors for gas treatment (6, 23, 26).

Various strategies to prevent clogging have been suggested. Diks et al. (6) added salt to the liquid phase and Weber and Hartmans (26) limited the amount of inorganic nutrients, in order to reduce the growth of biomass. The disadvantage of these methods is that often not only the biomass formation rate, but also the biodegradation rate is limited. The use of nitrate instead of ammonium, slightly reduced the biomass yield of propene-degraders, without affecting the biodegradation rate (Chapter 4). This phenomenon was also observed in trickle-bed reactors removing toluene (23). So, besides preventing nitrification, the use of nitrate as nitrogen source also has a beneficial effect by decreasing the biomass yield.

Removal of biomass to prevent clogging, has been tested in trickle-bed reactors by washing with strong alkali (26) and by backwashing (23). In hollow-fibre bioreactors we were able to prevent clogging by applying a very high liquid velocity in the fibres. However, irrespective of the liquid velocity in the fibres, the propene degradation rate of the hollow-fibre reactors gradually decreased to approximately half of the maximum value.

Apparently, clogging of the fibres is not the only factor resulting in a decrease in reactor performance. A possible explanation could be the inactivation of biomass close to the membrane. If biomass close to the membrane inactivates and is not replaced by new cells, it will hamper the transfer of pollutants to the active biomass further away from the membrane. Other possible causes are a lack of nutrients, membrane modification, insufficient humidification of the biomass close to the membrane, or the accumulation of toxic products in the biofilm.

Biofilm monitoring

In order to identify the cause of the decreasing biofilm performance, a method is required with which the activity of the biofilm can be assessed. For this purpose we chose a fluorescent probe, 5-cyano-2,3-ditolyl tetrazolium chloride (CTC). This compound is reduced by the respiratory chain of actively respiring bacteria to form a fluorescent product and is thus specific for viable cells. Since fluorescence microscopy is very sensitive, individual respiring cells in a biofilm should be detectable after staining with CTC (27). The back-side cover of this thesis shows a phase-contrast micrograph *(right side)* and an epifluorescence micrograph *(left side)* of a propene-oxidizing biofilm grown in a hollow-fibre and stained with CTC. As can be seen in the epifluorescence micrograph the staining is most intensive close

to the membrane, suggesting that the most active cells are present there. This is as expected, since the supply of carbon source and oxygen are greater close to the membrane. A layer of dead cells close to the membrane was not visible in this biofilm.

As a control we tested the reduction of the same probe after 24 hours of starvation. After such a 24 hours starvation period, hardly any propene-degrading activity can be found in suspended *Xanthobacter* Py2 cells (unpublished results). With biofilms, however, there was barely a difference between the staining of fresh and starved biofilms. Moreover, the addition of propene during the staining did not significantly intensify the fluorescence, indicating that the energy-status of cells in the biofilm, as determined with CTC, does not depend on the propene degradation rate.

From these experiments it can be concluded that the assesment of the respiratory chain activity using the fluorescent probe CTC, can give information on the viability of the cells, but it is by no means an indication of propene-biodegradation in biofilms. In the absence of propene, the cells in a biofilm possibly gain energy from the oxidation of extracellular compounds or cell debris.

To determine the location of propene-degrading cells, a method is required that is specific for the propene-degrading enzymes, e.g. the propene monooxygenase. A very elegant method would be to use a dye that is converted into a fluorescent product by this enzyme. In this way the cells degrading propene can be detected very specifically. We tested methoxyresorufin, that is converted into a fluorescent product by various monooxygenases (15). Fresh *Xanthobacter* Py2 cells, however, did not show any activity with this probe.

As an alternative to enzyme activity, messenger-RNA (mRNA) could be detected. mRNA is present in the cell only when the corresponding protein is being synthesized and mRNA is extremely unstable. By detecting mRNA encoding the propene monooxygenase or the epoxidase, the activity of the cell can be assessed very sensitively. Since the genes of the propene monooxygenase and the epoxidase of *Xanthobacter* Py2 have been cloned and sequenced (24, 28), this technique seems feasible for *Xanthobacter* Py2. The detection of mRNA to assess induction has been studied extensively in animal and plant tissues (11). Unfortunately, the bacterial cell wall and the high activity of RNA-degrading enzymes (RNases) in bacteria complicate the application of this technique to immobilized bacterial cells (10).

Membrane bioreactors for biofilm cultivation

Most techniques for biofilm analysis require cutting, fixation, and staining of the biofilm, before samples can be observed. By removing the biofilm from its substratum the biofilm may be disrupted and artefacts introduced. Membrane material, however, can easily be sectioned and processed along with the attached biofilm. Hence, biofilms grown on membranes can be processed relatively easily.

Membranes are also suitable for the cultivation of biofilms that have a high oxygen demand. Since there is no layer of water between the gas phase and the biofilm, oxygen transfer rates in membrane bioreactors are very high (Chapter 3). In addition, the membrane bioreactor might be used for the cultivation of biofilms in the presence of counter gradients (1, 4, 25). Summarizing it can be said that membrane reactors are not only promising as bioreactors for treatment of specific waste gases, but can also form useful tools in biofilm research.

Removal of highly chlorinated pollutants

Apart from biofilm research and the removal of poorly soluble pollutants from air, the membrane bioreactor might also be used for the removal of highly chlorinated hydrocarbons, such as trichloroethylene, from waste streams. TCE can be degraded continuously by *Xanthobacter* Py2 in the presence of the growth substrate propene (Chapter 6) and similar studies have been performed with other strains and their respective growth substrates (14, 16). The growth substrates required for cultivation of these strains generally have a high volatility and direct contact between the TCE-contaminated air stream and growth substrate would therefore lead to loss of growth substrate and contamination of the air to be cleaned. In a membrane bioreactor the waste gas stream and the volatile growth substrate can be kept apart.

Unfortunately, I have not been able to test the membrane bioreactor for continuous degradation of both TCE and propene. A similar concept was, however, recently demonstrated with TCE-containing wastewater by Aziz et al. (2). In their study a TCE-degrading culture of methanotrophs was circulated from the hollow-fibre contactor to a separate reactor, where methane and oxygen were supplied. In the hollow-fibre contactor the TCE diffused from the contaminated wastewater through the membrane to the other liquid compartment where it was degraded by the methanotrophs.

Ensign et al. (8) recently studied the induction of the propene monooxygenase required for TCE degradation. They concluded that TCE might be degraded by *Xanthobacter* Py2 without the addition of volatile and competitive growth substrates, since various chlorinated alkenes were able to induce the monooxygenase. The inducing substrates include vinyl chloride and 1,2-dichloroethene, but not trichloroethene. A mixture of TCE and the inducing substrates vinyl chloride and 1,2-dichloroethene, however, could be indeed degraded by glucose-grown cells. If the regulation of the propene monooxygenase production is elucidated further, it might be possible to continuously degrade TCE by using a culture of *Xanthobacter* Py2 that is simply growing with glucose and an inducing substrate. However, a reactor that is fed with an easily degradable substrate like glucose, will be vulnerable to contamination, expecially since the infections do no suffer from the toxic effects of TCE-degradation.

With other TCE-degrading strains continuous TCE-degradation in the absence of volatile substrates was pursued by constructing strains in which the monooxygenase was expressed constitutively (13, 18, 21).

Another possible method to degrade TCE without the addition of volatile growth substrate, is the combined anaerobic/aerobic membrane bioreactor proposed by Parvatiyar et al. (20). The liquid compartment of this membrane bioreactor was kept anaerobic by the addition of large amounts of acetate. According to the model that the authors propose, TCE is initially dechlorinated in the anaerobic zone of the biofilm. The degradation products are then degraded further in the aerobic zone (Chapter 2). In this publication, however, no experimental evidence is presented for the production of intermediates or for the existence of both an aerobic and an anaerobic zone in the biofilm. Nevertheless, this is the first study, in which TCE was shown to be continuously removed from air, without the addition of large amounts of a volatile growth substrate, like e.g. methane or propene.

In addition to TCE, perchloroethene (PCE) was recently found to be degraded under anaerobic conditions by a facultatively aerobic bacterium (22). As recently as 1993, Dolfing et al. (7) concluded in their review on the biological degradation of chlorinated hydrocarbons, that there are no efficient process technologies for transfer of contaminants from the gas phase to an anaerobic compartment. The development of membrane bioreactors with both an anaerobic and an aerobic zone, and the isolation of facultatively aerobic strains that are able to dechlorinate, introduces new possibilities for the removal of pollutants that until now are considered to be beyond the reach of (aerobic) biological waste gas treatment.

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Summary

This thesis describes the design and testing of a membrane bioreactor (MBR) for removal of organic pollutants from air. In such a bioreactor for biological gas treatment pollutants are degraded by micro-organisms. The membrane bioreactor is an alternative to other types of bioreactors for waste gas treatment, such as compost biofilters and bioscrubbers. Propene was used as a model pollutant to study the membrane bioreactor .

A membrane bioreactor for waste gas treatment consists of a gas and a liquid compartment, separated by a membrane. Gaseous pollutants diffuse through the membrane and are consumed by microorganisms present in the liquid phase. The organisms are supplied with water and inorganic nutrients via this liquid phase. Various membrane bioreactors described in the literature are reviewed in Chapter 2. In the work presented in this thesis, microporous hydrophobic material was selected because of its low mass transfer resistance and the availability of both sheets and fibres. For the removal of propene from air the mass transfer resistance of this type of membrane was found to be negligible (Chapter 3).

The propene-degrading bacterium *Xanthobacter* Py2 was shown to form biofilms in membrane bioreactors. Continuous propene removal by biofilms of *Xanthobacter* Py2 was demonstrated in both flat sheet reactors and hollow-fibre reactors. In both configurations the biofilms are situated on the membrane in the liquid phase. Propene consumption rates could be described quite accurately with the computer programme BIOSIM, that describes simultaneous diffusion and reaction in a biolayer (Chapter 3).

During continuous operation of hollow-fibre reactors at inlet concentrations of 0.5 to 6 gram propene per m^3, the propene conversion decreased after several weeks (Chapter 4). Clogging of the fibres by excess biomass formation and acidification due to ammonium oxidation, were identified as possible causes. However, when both clogging and ammonium oxidation were prevented, the propene conversion still decreased in time.

Apparently other factors than clogging and nitrification affect the long-term performance of biofilms of *Xanthobacter* Py2, growing in an MBR. These factors might be identified with new methods for biofilm analysis, which allow the localization of activity within the biofilm.

According to the Dutch emission standards, hydrocarbons such as propene, in off-gas have to be reduced to less than 150 mg m^{-3}. In Chapter 5, two propene-degrading strains were compared for their ability to degrade such low concentrations of propene and the faster growing strain, *Xanthobacter* Py2, was selected. At a concentration of 300 to 600 mg m^{-3} in the gas phase, a 20 days start-up period was required for biofilm formation. Once the biofilm had been established, the amount of active biomass adapted to the amount of propene available within several days. Propene could be removed continuously from air at a concentration of 15 to 50 mg m^{-3} in the gas phase without supplying other organic nutrients to the microbial population (Chapter 5).

Besides the removal of poorly water soluble pollutants like propene, the membrane bioreactor is also suitable for the removal of pollutants that result in acidification, such as chlorinated hydrocarbons. Therefore, in Chapter 6 the biodegradation of trichloroethene (TCE) by *Xanthobacter* Py2 was tested during growth on propene in a stirred vessel. The aerobic biodegradation of TCE is difficult because of toxic intermediates that are formed. With *Xanthobacter* Py2 continuous cometabolic degradation of TCE was shown to be feasible with concentrations up to 206 μM in the liquid phase. The amount of TCE that could be degraded, depended on the TCE concentration and ranged from 0.03 to 0.34 grams of TCE per gram of biomass.

Membrane bioreactors for gas-liquid contact have several potential applications. They are suitable for the removal of poorly soluble pollutants from air because of their large gas-liquid interface and small mass transfer resistance. Especially if biodegradation of a poorly soluble pollutant results in acidification, the membrane bioreactor might be a unique tool, since the acidic product can be removed via the liquid phase. Other applications might be the removal of highly chlorinated hydrocarbons from air by an aerobic or a combined anaerobic/aerobic process, as was recently suggested in literature. Membrane bioreactors may also be useful tools in biofilm research, because of easy handling and processing of biofilm samples, excellent oxygen transfer properties and the possibility to apply counter gradients.

Samenvatting

Dit proefschrift beschrijft een membraanbioreactor (MBR), waarmee organische vervuiling uit lucht verwijderd kan worden. In een dergelijke reactor voor biologische luchtreiniging worden de vluchtige stoffen afgebroken door microorganismen. De hier beschreven membraanbioreactor is een alternatief voor reeds bestaande typen bioreactoren, zoals het compostbiofilter en de biowasser. Propeen is in het hier beschreven onderzoek gebruikt als modelverbinding om de MBR te testen.

In een MBR voor biologische gasreiniging vormt het membraan het grensvlak tussen het lucht- en het watercompartiment. Vluchtige stoffen diffunderen door het membraan heen naar de waterfase en worden daar afgebroken door microorganismen. De verschillende typen membraan-reactoren die in de literatuur beschreven zijn, staan samengevat in hoofdstuk 2. In het onderzoek dat beschreven is in dit proefschrift, is gebruik gemaakt van microporeus hydrofoob membraanmateriaal, omdat dit een lage stoftransportweerstand heeft en omdat het verkrijgbaar is in de vorm van velletjes en vezels. Tijdens de verwijdering van propeen is de weerstand van dit membraan verwaarloosbaar klein (hoofdstuk 3).

Propeen kan continu verwijderd worden uit lucht door een biofilm van *Xanthobacter* Py2, in zowel vlakke-plaat-reactoren als holle vezels. In beide typen reactoren bevindt de biofilm zich in de vloeistoffase, direct aan het gasgevulde membraan. De biofilm wordt vanuit de vloeistoffase voorzien van water en anorganische nutriënten, terwijl het gasvormige propeen vanaf de gaszijde wordt aangevoerd. De snelheid van propeenconsumptie kan tamelijk nauwkeurig worden beschreven met behulp van het programma BIOSIM, dat gelijktijdige diffusie en reactie simuleert (hoofdstuk 3).

Toen een holle-vezel-reactor continu bedreven werd bij een propeenconcentratie van 0.7 tot 6 g per m^3, daalde na enkele weken de propeenconversie (hoofdstuk 4). Dit leek te wijten aan verstopping van de vezels door de vorming van een overmaat aan biomassa of aan verzuring door de oxidatie van ammonium. Toen echter verstopping en ammoniumoxidatie verhinderd werden, liep de propeenconversie na verloop van tijd alsnog terug. Kennelijk zijn op de lange duur andere

factoren dan verstopping en verzuring van belang. Deze factoren zou men kunnen achterhalen door biofilms gedurende langere tijd nauwkeurig te volgen. Hiervoor zijn echter nieuwe analysetechnieken nodig.

De Nederlandse norm voor de uitstoot van koolwaterstoffen in afgas is 150 mg m^{-3}. In hoofdstuk 5 hebben we daarom twee bacteriestammen vergeleken bij propeenconcentraties rond die norm van 150 mg m^{-3}. Van de twee stammen is de snelst groeiende, *Xanthobacter* Py2, gekozen voor nader onderzoek. Bij een concentratie van 300 tot 600 mg m^{-3} propeen in de gasfase, vergde de ontwikkeling van een biofilm 20 dagen. Toen de biofilm eenmaal gevormd was, bleek de hoeveelheid actieve biomassa zich aan te passen aan de beschikbare hoeveelheid propeen. Continue afbraak van propeen bleek mogelijk bij concentraties van 15 to 50 mg m^{-3}, zonder toevoeging van andere energiebronnen dan propeen.

Behalve voor de verwijdering van slecht oplosbare verbindingen, zoals propeen, kan de membraan bioreactor in principe ook gebruikt worden voor de verwijdering van vluchtige stoffen die tot verzuring leiden, zoals gechloreerde koolwaterstoffen. Daarom is in hoofdstuk 6 de afbraak van trichlooretheen (TCE) onderzocht. *Xanthobacter* Py2 bleek TCE te kunnen afbreken tijdens groei op propeen in een geroerd vat. Continue biologische verwijdering van TCE uit lucht is moeilijk, omdat er giftige(tussen)producten gevormd worden tijdens de aerobe afbraak. Met *Xanthobacter* Py2 kon TCE continu afgebroken worden tot de hoogst gemeten concentratie van 206 μM in de waterfase. De hoeveelheid die kon worden omgezet, hing af van de concentratie en bedroeg 0.03 to 0.34 g TCE per g biomassa.

Membraanbioreactoren kunnen mogelijk in verschillende gebieden toegepast worden. Zij zijn geschikt voor de verwijdering van slecht-water-oplosbare vluchtige stoffen uit lucht, omdat in een MBR een groot gas-vloeistofoppervlak gecreëerd kan worden en de stoftransportweerstand laag is. Vooral als de afbraak van een slecht oplosbare verbinding leidt to verzuring, is de MBR uniek, omdat de zure producten via de waterfase afgevoerd kunnen worden. Een andere toepassing is mogelijk de verwijdering van sterk gechloreerde koolwater-stoffen uit lucht, door een aeroob of een gecombineerd anaeroob/aeroob proces, zoals recentelijk is de literatuur voorgesteld werd. Membraanbioreactoren kunnen ook goede diensten bewijzen in het biofilm onderzoek. In een MBR is de zuurstoftoevoer uitstekend, er kunnen gradiënten aangelegd worden en bovendien zijn biofilms op een membraan makkelijk te hanteren en te bewerken.

Curriculum vitae

Martine Wilhelmina Reij werd op 16 mei 1967 geboren te Brunssum. In 1985 behaalde zij het gymnasiumdiploma met lof aan het Bisschoppelijk College te Sittard en begon daarop aan de studie levensmiddelentechnologie bij de Landbouwuniversiteit te Wageningen. Met de afstudeervakken proceskunde en microbiologie en een stage bij de afdeling Biological Sciences van de University of Calgary (Canada), studeerde zij in juni 1991 af. Daarna werd Martine assistent-in-opleiding bij de Sectie Industriële Microbiologie van de Landbouwuniversiteit te Wageningen en deed onderzoek aan membraan-bioreactoren voor biologische gasreiniging. Van oktober 1995 tot april 1996 werkte ze bij diezelfde sectie als toegevoegd onderzoeker, in opdracht van Stork Comprimo. Sinds augustus 1996 werkt ze als docent milieutechnologie bij de Agrarische Hogeschool in Den Bosch.

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Photographs shown on the covers

Front cover

The front cover shows the two types of membrane bioreactors described in this thesis: a hollow-fibre module *(left)* and a flat sheet membrane *(right)*. The cross-section of the hollow-fibre module shows biofilms of yellow propene-oxidizers inside the hollow fibres.

The picture on the right shows a biofilm of the yellow bacterium *Xanthobacter* Py2 grown on a flat sheet of white membrane material.

Back cover

The back cover of this thesis shows a phase-contrast micrograph *(right)* and an epifluorescence micrograph *(left)* of the same biofilm sample. This biofilm was grown with propene in a hollow-fibre bioreactor for three months. The biofilm was stained with CTC, which is a probe for respiratory chain activity. Biofilms were then frozen in liquid nitrogen and samples with a thickness of 6 µm were cut in a cryostat at -20°C.

The sample consists of a piece of membrane material with a biofilm attached at the upper side. In both photographs the white arrows indicate the interface between the biofilm and the membrane. In the epifluorescence micrograph it can be seen that the membrane is not stained by CTC. The staining of the biofilm is more intensive close to the membrane, suggesting that the most active cells are situated close to the membrane.