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# Gas-phase methyl ethyl ketone biodegradation in a tubular biofilm reactor: microbiological and bioprocess aspects

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**Abstract**

A novel type of bioreactor was designed to clean VOCs-containing air. The operation of this reactor consists in mixing the polluted gas and a mist of nutrient solution in the presence of microorganisms in order to maximize contact and transfer between gas, liquid and microorganisms and to promote the degradation kinetics and the relative removal efficiency of the pollutant. A bacterial consortium acclimatized to MEK and containing a preponderance of *Alcaligenes denitrificans* was established under non-axenic conditions. On the tubular reactor's glass walls, a continuous biofilm was developed. This biofilm was rapidly contaminated by two fungi able to degrade MEK: *Geotrichum candidum* and *Fusarium oxysporum*. Their abundance in the reactor is probably linked to the acidic conditions inside the biofilm and to their broader tolerance for low pH values concomitant with MEK degradation. In the reactor, a maximum volumetric degradation rate of 3.5 kg MEK/$m^3_{reactor}$·d was obtained for a relative removal efficiency of 35%, whereas the latter was maintained at 70% for more modest applied loadings of 1.5 kg MEK/$m^3_{reactor}$·d. In liquid batch cultures, a biomass originating from the biofilm was able to degrade 0.40 $g_{MEK}/g_{DCW}$·h at the optimal pH of 7. A regular cycle of detachment-recolonization was observed during the operation of the bioreactor. The maximal degradation activity was obtained with a thin biofilm and was not increased as the biofilm grew in thickness. The overall degradation rate of the process did not appear to be limited by the diffusion of oxygen inside the biofilm. Over short periods of time, the MEK transfer from the gaseous phase to the biofilm was neither affected by the presence of the mist nor by the wetting of the biofilm. A better control of the biofilm pH led to improved performance in terms of removal rate but not in terms of relative elimination efficiency.

*Abbreviations:* MEK – methyl ethyl ketone; DCW – dry cell weight; VOCs – volatile organic compounds

## Introduction

Odors and air pollution stemming from volatile organic compounds (VOCs) are becoming an increasingly serious problem. Under aerobic conditions VOCs can be eliminated by suitable microorganisms, since these compounds can serve as carbon and energy sources for microbial metabolism. In this way, the gaseous pollutants are not transferred in concentrated form to another phase, but are converted to harmless oxidation products such as carbon dioxide. In general terms, the biological elimination of VOCs from gaseous streams can be described by a mechanism consisting of transport into a liquid or solid phase followed by microbial degradation. Several bioprocessing systems are currently in use, distinguishable by the behavior of the liquid phase (moving or stationary) and of the microorganisms (freely dispersed or immobilized). Based on these criteria, waste gas biotreatments include biofilters, bioscrubbers and biotrickling filters (beds) (Ottengraf 1986; Diks & Ottengraf 1991; De Heyder et al 1992; Groenestijn & Hesselink 1993; Andrews &

Noah 1995). Biofilters are packed beds of porous solid support particles (compost, peat, bark, soil etc.) that incorporate a microbial population capable of degrading the gas compounds blown through the bed. The filter media and the microorganisms are presumed to be surrounded by a thin liquid (aqueous) film into which the VOC partitions, is fixed on the solid material by adsorption and becomes available for biodegradation. Key process variables here include the type and size of the particles and the amount of humidity in the bed that can affect the molecular diffusion of the gaseous pollutant before its degradation. Bioscrubbers are either conventional bubble columns in which the waste gas is blown through a liquid phase with dispersed microbial growth or, more specifically, a separate chamber through which absorbing liquid medium recirculated from a well-mixed suspended-growth bioreactor is brought into contact with the gaseous stream. Gas-to-liquid mass transfer considerations in combination with the intrinsic biodegradation kinetics guide the process operation. Finally, in a trickling bed, the contact column can be packed with support particles for the microorganisms to attach and form a biofilm. The waste gas is contacted with this biofilm, which, however, is often percolated by liquid medium. The VOC can thus be transferred first to the liquid phase and then to the biofilm, where it is eventually degraded. In contrast to the typically slow diffusional movement of the gaseous component in a biofilter, the performance of the biotrickling process may be influenced by the rapid turbulent mixing of the VOC that can occur within the falling liquid film, so that the biolayer in this type of reactor can be considerably thicker before mass transfer limitations become dominant. These three technologies are specifically suited for different classes of gaseous pollutants and have all advantages and disavantages. On the basis of Henry's constant (H), biofiltration is considered to be particularly efficient for poorly soluble gaseous substances ($1 < H < 10$), bioscrubbing for very soluble substances ($H < 0.01$) and biotrickling filtration for intermediately soluble ($0.01 < H < 1$) and acidifying compounds (Diks et al. 1994; Kirchner et al. 1996). On the other hand, bioscrubbers and biotrickling filters are more expensive and more difficult to construct, operate and maintain than biofilters, but require smaller space than the latter (Groenestijn & Hesselink 1993). Nonetheless, most designs in the area of vapor-phase biotreatment are still empirical and not based on a set of standardized guidelines derived from the relevant biological and physicochemical phenomena (Andrews & Noah 1995).

As a general rule and with the exception of pollutants requiring specific microbial strains for degradation, the bioreactors used to treat VOCs are considered as black boxes, taking into account mostly mass transfer phenomena rather than microbiological aspects. Therefore, the microorganisms used are generally undefined, taken from a wastewater treatment plant, which has been acclimatized to the pollutant. Moreover, the carrier materials chosen to support biomass development are often compact and offer a high specific exchange surface area with air. Under these conditions, it is nearly impossible to have direct access to the microorganisms in the reactor to study their behavior and to influence their development.

Recently a novel tubular biofilm reactor has been developed to overcome the disadvantages over the above-mentioned gas biotreatment systems. The operating principle is the mixing of the VOC-containing gas together with a nutrient solution through an atomizer to obtain an optimal contact between gas, liquid and microorganisms that grow as a biofilm on the tubular reactor's inner wall surface (Thalasso et al. 1991; 1994). The atomizer nozzle promotes exceptionally high gas-to-liquid mass transfer rates (Thalasso et al. 1995) and allows the independent input and control of the substrate gas feed and of the aqueous nutrient medium feed to form a thin mist cone that is being 'shot' against the biofilm. The extremely low input of water, together with the high turbulence of the atomizing mechanism and the absence of poorly defined packing materials has contributed to the development of a new generation of biofilters, with fewer design and operational unknowns. The reactor was shown to efficiently degrade methanol in the gas phase with a specific exchange surface of only 27 $m^2/m^3{}_{reactor}$ which corresponded to the surface of the reactor walls. Up to 46 kg methanol/$m^3{}_{reactor}$·d was degraded with an efficiency of 100% (Thalasso 1993). There were no problems of clogging. Moreover the biofilm formed could be characterized (Thalasso et al. 1996).

This reactor type has been used to evaluate the vapor-phase removal of MEK, a readily biodegradable (Price et al. 1974) and odorous substrate, easily soluble ($H = 5.10^{-3}$at 35 °C). MEK, a widely used industrial solvent and a major component of paints, varnishes, inks, etc. served as a model VOC pollutant for this study.

Surprisingly, few reports deal with the pathways for the degradation of ketones (Deshusses 1994). Their catabolism appears to be analogous to that of n-alkanes, whose main degradation pathways involve an initial oxidative attack of the terminal methyl groups by

monooxygenases, leading to the formation of fatty acids, which in turn can be further metabolized for energy generation via $\beta$-oxidation (Singer & Finnerty 1984; Rittmann et al. 1994). A side or co-metabolic reaction in n-alkane degradation is subterminal oxidation, in which a methylene carbon is hydroxylated, producing a secondary alcohol to be further oxidized to a ketone by an alcohol dehydrogenase (Singer & Finnerty 1984). Monooxygenases can insert an oxygen atom between the carbonyl carbon and an adjacent methylene carbon atom to yield an ester which can be cleaved by an esterase into the corresponding carboxylic acid and primary alcohol (Taylor et al. 1980; Singer & Finnerty 1984; Rittmann et al. 1994). Depending on the physicochemical and microbial ecological context, these products can either accumulate or be further degraded (Taylor et al. 1980). The degradation of MEK may proceed, at least transiently, via formation of acetic acid and ethanol.

This study is aimed at investigating the degradation of MEK by a bacterial consortium in liquid batch culture, to adapt the microorganisms to increasing MEK concentrations, and to apply the novel gas-phase reactor to the non-axenic treatment of air contaminated by MEK.

## Materials and methods

### *Tubular biofilm bioreactor design and operation*

The bioreactor consisted of a segmented Pyrex® glass tube of 0.147 m in internal diameter and 1.12 m in length (Figure 1). Four 10-cm long glass cylinders forming the body of the reactor were joined by O-shaped rings and bridles to guarantee perfect gas tightness. The top of the reactor was a 40 cm long unsegmented tube. Beneath the body of the reactor, there was a 20 cm long cylinder and a 12 cm long terminal piece. This module was equipped with two exit tubes permitting the separation of effluent gas and liquid for mass balances around the bioreactor (see below). All construction materials were inert towards MEK (Teflon®, PVDF, stainless steel, etc.). The bioreactor system was housed in a constant room environment, where the temperature of operation was 35 °C. Although there are no definitive data in the literature concerning the optimum temperature for MEK biodegradation, this choice was made in view of the technological interest it represents, since treatment of gaseous effluents in situ may involve either an emission cooled down to 35 °C with process cooling water or, alternatively, the waste VOC may represent an emission from an otherwise useful gas stream in the process of being steam heated for an in-plant application.

At the top of the bioreactor, a liquid medium was introduced in the form of a mist via a pneumatic atomizing nozzle (Lechler®, model 156.330.16.16, Fellbach, Germany). The volumetric liquid flow rate ($Q_{v,l}$) was 0.632 $m^3{}_l/m^3{}_{reactor}$·d. The liquid medium was an aqueous solution (distilled water) of the following compounds: $Na_2HPO_4.2H_2O$ 1.78 g/l, $KH_2PO_4$ 0.32 g/l, $(NH_4)_2SO_4$ 3 g/l, $MgSO_4.7H_2O$ 0.05 g/l, EDTA $Na_2.2H_2O$ 10 mg/l, $FeSO_4.7H_2O$ 5 mg/l, $MnCl_2.4H_2O$ 1.22 mg/l, $CoCl_2.6H_2O$ 0.4 mg/l, $ZnSO_4.7H_2O$ 0.25 mg/l, $CuSO_4.5H_2O$ 0.2 mg/l, $CaCl_2.2H_2O$ 1 mg/l, $Na_2MoO_4.2H_2O$ 0.2 mg/l, biotin 0.02 mg/l, choline-Cl 1 mg/l, Ca-pantothenate 0.5 mg/l, inositol 2 mg/l, nicotinic acid 0.5 mg/l, pyridoxine-Cl 0.5 mg/l, thiamine-HCl 0.5 mg/l, *p*-aminobenzoic acid 0.2 mg/l, cyanocobalamin 0.01 mg/l and folic acid 0.1 mg/l (Thalasso 1993; Thalasso et al. 1996). The pH of this medium was 7.3. Ninety percent of the total volumetric air flow ($Q_{v,g\ total}$ = 1,263 $m^3{}_g/m^3{}_{reactor}$·d) entering the reactor was used for liquid atomization and 10% was used for volatilization of MEK. This partial air flow went through a pipe in which pure liquid MEK (Acros 14.967.29, Pittsburgh, PA, USA) was introduced via a precision feeding pump (ProMinent®, model 'mikro g/5a 400150TT', Brussels, Belgium), allowing its complete stripping.

### *Bioreactor mass balances*

Mass balances around the bioreactor were carried out by sampling influent and effluent gas and liquid. The effluent gas was channelled towards a 0.5-l gas sampling bulb (Alltech®, model 7011, Deerfield, IL, USA) whose taps were closed after flushing for a period of ten minutes. In the same way, the influent gas was sampled from the air-stripping pipe through the use of appropriate valves (see Figure 1). The influent and effluent gas flow rates were measured with suitable gas flow meters (Schlumberger, models 1 and G4, Dordrecht, The Netherlands). The concentration of MEK in the liquid and gas phase was determined by gas chromatography (see *Analytical methods*).

The mass balance equation was expressed as follows (see the list of symbols at the end of the article):

$$r_v = ([MEK]_{g,in}.Q_{v,g,in}) - \{([MEK]_{g,out}.Q_{v,g,out}) + ([MEK]_{l,out}.Q_{v,l,out})\} \quad (1)$$

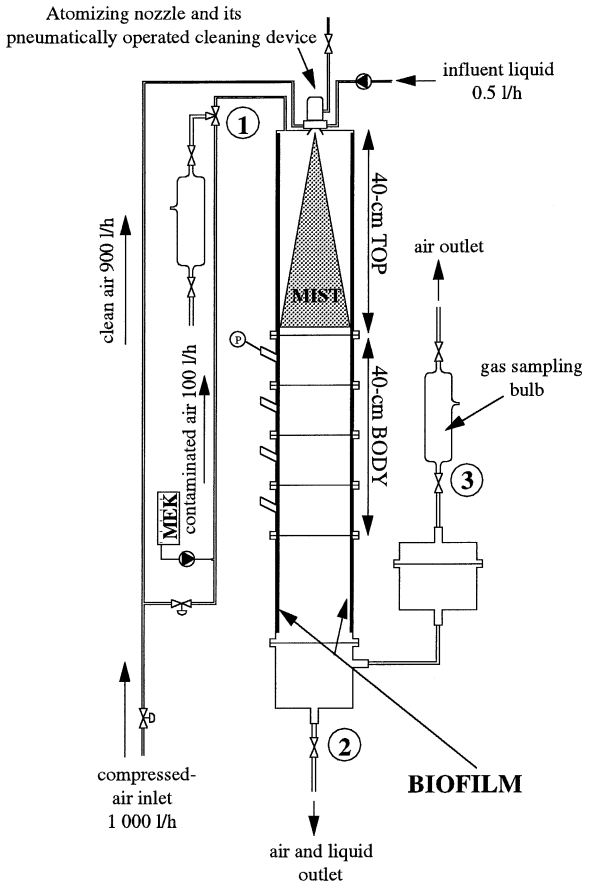


*Figure 1.* Experimental set-up of the 19-l DTB bioreactor: 1, sampling point for influent artificially polluted air; 2, sampling point for effluent liquid; 3, sampling point for effluent gas. For more details, see text.

$$r_v = B_{v,g,in} - (B_{v,g,out} + B_{v,l,out}) \qquad (2)$$

$$R_{\%} = 100.r_v/B_{v,g,in} \qquad (3)$$

In Eq. 1, $r_v$ is the volumetric MEK degradation rate (kg/m$^3{}_{reactor}$·d) and the product of concentration ([MEK]) and volumetric flow rate ($Q_v$) represents the volumetric load (symbolized by $B_v$ in Eq. 2). The relative removal efficiency ($R_{\%}$) is the ratio between the volumetric MEK degradation rate and the inlet volumetric MEK load, and is expressed as a percentage (Eq. 3).

In the absence of microorganisms, the range of difference in measurement between the influent and effluent loads was ± 10%. When the inlet MEK load was changed, two hours were necessary for the reactor to reach a new equilibrium.

*Inoculum*

The inoculum was a sample of the biofilm which had previously colonized the bioreactor with methanol as a model pollutant (Thalasso 1993). This mixed culture sample was rapidly acclimatized to MEK by transferring it to a liquid batch culture containing progressively higher concentrations of MEK (0.3, 1 and 3 g/l).

*Kinetic studies in liquid batch cultures and related techniques*

To establish biodegradation parameters in batch cultures, 1.2-l air-tight bottles were equipped with a sidearm allowing the reading of the optical density on a Klett-Summerson colorimeter (red filter). Therefore, the biomass growth could be followed without having to open the flasks given the volatility of MEK. Optical density (OD) was converted into dry cell weight. For this, a standard curve was established by serial dilutions of a homogeneous concentrated microorganism suspension. OD of the different dilutions were measured and the samples dried at 105 °C.

In the bottles, the liquid volume was limited to 50 ml in order to provide enough oxygen to the biomass. This volume was established based on the stoichiometry of the following theoretical equation for MEK degradation, where $C_5H_7NO_2$ is the assumed mean elemental composition of the biomass:

$$C_4H_8O + \tfrac{2}{5}NH_3 + \tfrac{7}{2}O_2 \longrightarrow \tfrac{2}{5}C_5H_7NO_2 + 2CO_2 + \tfrac{16}{5}H_2O \qquad (4)$$

The assumption of MEK mineralization in this equation was supported by the measurements of MEK, biomass and carbon dioxide, together with the lack of accumulation of any carbon-containing metabolite in the medium.

The time course of the MEK concentration in the liquid phase was followed by gas chromatography after taking 0.5-ml samples through a rubber septum and adding an internal standard (see *Analytical methods*). Batch cultures were agitated on a gyratory shaker at 150 rpm and 35 °C. The batch culture medium was the same as the one used in the reactor except that the buffer concentration was two-fold higher to guarantee a stable pH.

At the start and the end of each degradation experiment, carbon dioxide and oxygen were measured in the gas and liquid phase (see *Analytical methods)*. At

the end of the degradation, potential acidic metabolites (acetic acid, formic acid, propionic acid, lactic acid, etc.) were sought by HPLC (see *Analytical methods*) and residual ammonium was measured by the distillation and titration method of Bremner (1965).

*Microbial counting and isolation*

Bacteria were counted by serial dilutions in a 0.9% NaCl solution or isolated by streaking and transfers on Plate Count Agar (PCA, Difco 0479, Detroit, MI, USA) with and without the following antifungal compounds: benlate® (Du Pont De Nemours, Bad Homburg, Germany) and actidione (Fluka 01810, Buchs, Switzerland), 0.1 g/l each. For yeasts and fungi, a solid medium (DYPA) was used. It was composed of dextrose (Merck 8346, Darmstadt, Germany) 20 g/l, yeast extract (Difco 0127, Detroit, MI, USA) 5 g/l, peptone (Merck 7214, Darmstadt, Germany) 10 g/l and agar (Fluka 05040, Buchs, Switzerland) 20 g/l with and without chloramphenicol (Aldrich 85.744, Steinheim, Germany) and tetracycline-HCl (Sigma T-3383, Saint Louis, MO, USA), 0.3 g/l each.

For biofilm studies, at least 100 g (wet weight) of biofilm were taken over a known area in the reactor, that was calculated geometrically over the various horizontal segments (zones) of the cylinder. To further refine the measurements of biofilm samples the zones were subdivided into radian quarters and percentages were estimated visually from each quarter for the totality of the zone examined. Given the variation of thickness due to biofilm heterogeneity (Zhang & Bishop 1994a; 1994b; Murga et al. 1995) the locations were randomized by assigning three or more of them in each zone of observation on a rotating basis. For the measurements of film thickness and microbial cell density, each time duplicate samples were taken from an approximate surface of 10 cm x 10 cm (the biofilm was cut and the surface evaluated). The biofilm mean thickness was calculated as the ratio between the volume of the biofilm, measured by saline (NaCl 0.9%) volume displacement in a 1-l cylinder, and the area over which this biofilm was taken. After putting the biofilm in suspension by agitation (about 100 g biofilm wet weight/l), serial dilutions were carried out for counting on specific solid media. Then, 100 g of the suspended biofilm were dried at 105 °C.

*Analytical methods*

The concentration of MEK in the liquid phase was determined by injecting 1 $\mu$l in an Intersmat gas chromatograph Model IGC121DFL (Chelles-les-Coudreaux, France) equipped with a glass column (2 m long, 3 mm internal diameter, 1/4″ external diameter) filled with Porapak Q 80-100 mesh as stationary phase (Alltech, Deerfield, IL, USA). Nitrogen was used as carrier gas. The temperature of the injector and the flame ionization detector was 220 °C. The analysis was carried out isothermally at 185 °C. Before injection, absolute ethanol (Merck 1.00983, Darmstadt, Germany) was added to the sample as internal standard.

MEK concentrations in the gas phase were measured by injecting 10 or 500 $\mu$l of gas with a suitable gas-tight syringe (Pressure-Lok®, Dynatech, A-2 series, Alltech catalogue numbers 050023 and 050033) into a Shimadzu gas chromatograph Model GC-14A (Kyoto, Japan) equipped with a CP-Sil 5CB capillary column (Chrompack 7685, 50 m long, 0.53 mm internal diameter, 5 $\mu$m film thickness, Middelburg, The Netherlands). The gas chromatograph was equipped with a flame ionization detector connected with a computing integrator (Shimadzu C-R4A). The analysis was carried out isothermally at 50 °C. The injector and detector temperatures were 270 and 285 °C respectively. Nitrogen was the carrier gas. The gas concentrations were computed from an external standard curve. The detection limit was 0.05 mg/l.

Carbon dioxide and oxygen in the gas phase were measured by gas chromatography using an Intersmat IGC120MB chromatograph (Chelles-les-Coudreaux, France) equipped with two columns in series (1/8″ external diameter), filled, respectively, with Porapak Q 50-80 mesh (3 m long column) and molecular sieve 13X 50-80 mesh (1.5-m long column), and with a 1.5-m long reference column filled with 10% SE 30 on Chromosorb PAW 80–100 mesh. Columns and stationary phases were purchased from Chrompack (Middelburg, The Netherlands). The injection (300 $\mu$l injection loop) and the analysis were carried out isothermally at 30 °C. The carrier gas was argon. The detector was a catharometer (50 mA, 145 °C). In the liquid phase, oxygen and total carbon dioxide were measured respectively by a polarographic oxygen probe and back titration after vacuum evaporation of carbon dioxide.

Organic acids were analysed by HPLC (Waters, Milford, MA, USA) equipped with a photodiode array detector Model Waters$^{TM}$996 UV and a column Supelcogel$^{TM}$GL-C610H-SP (Supelco, Bellefonte, PA, USA). The mobile phase was 0.1% phosphoric acid. The flow rate was 0.5 ml/min and 10 $\mu$l of sample were injected and detected at 210 nm. The specific organic acids that could be detected includ-

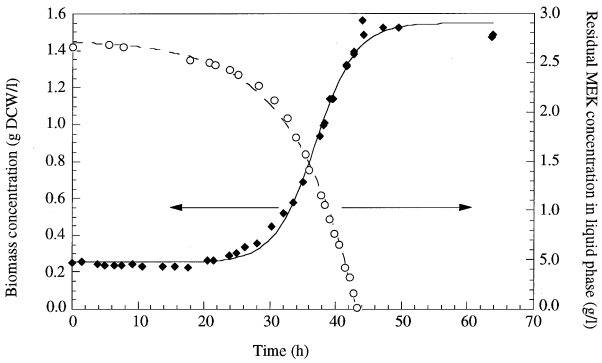


*Figure 2.* Growth (♦) and degradation (○) curves for the bacterial consortium used to inoculate the DTB bioreactor. The initial MEK concentration in the batch culture was 3 g/l. The solid line is the best-fitted sigmoidal growth curve and the dashed line is the best-fitted logarithmic degradation curve. The points are the experimental data.

*Table 1.* Experimental and theoretical values of microbial growth and degradation parameters in liquid batch experiments for an initial MEK concentration of 3 g/l.

| Parameter | Experimental value | Theoretical value |
|---|---|---|
| $\mu_{max}$[$h^{-1}$] | 0,16 | – |
| $q_{smax}$[$g_{MEK}/g_{DCW}$.h] | 0,26 | – |
| $Y_{X/MEK}$[$g_{DCW}$/g] | 0,43 | 0,70 |
| $Y_{O2/MEK}$[mole/mole] | 3,30 | 3,5 |
| $Y_{CO2/MEK}$[mole/mole] | 1,80 | 2 |
| $Y_{N/MEK}$[mole/mole] | 0,35 | 0,40 |
| $Y_{X/O2}$[$g_{DCW}$/g] | 0,30 | 0,45 |
| $Y_{X/CO2}$[$g_{DCW}$/g] | 0,39 | 0,57 |
| $Y_{X/N}$[$g_{DCW}$/g] | 6,23 | 9,00 |

Theoretical values are calculated from Eq. 4 (see Text). Symbols: $\mu_{max}$, maximum specific growth rate; $q_{max}$, maximum specific degradation rate; Y, yields; DCW, dry cell weight.

ed: acetic, adipic, ascorbic, benzoic, butyric, citric, formic, fumaric, isobutyric, isocitric, lactic, maleic, malic, malonic, oxalic, phytic, propionic, quinic, shikimic, succinic and tartric acid.

## Results and discussion

### *Inoculum characterization*

After the acclimation period, the microbiological composition of the inoculum destined for the tubular bioreactor (called DTB for 'Dry Tubular Bioreactor') was qualitatively determined based on colonies of culturable bacteria. Only four bacterial strains could be cultured out of the inoculum, allowing the possibility that additional microorganisms may have existed in the inoculum which could not be cultured in isolation on the solid media used. Among these four recovered strains one was dominant on PCA solid medium (92% of total colonies) and identified as *Alcaligenes denitrificans*. Two other bacteria were gram-negative rods and were not identified. The fourth one produced orange colonies on PCA, was a gram-positive and morphologically variable (filaments, cocci or short rods) microorganism and appeared to belong probably to Nocardioforms. These four bacteria were capable of growing together in the culture medium with MEK serving as their only source of carbon and energy.

The biodegradation performance of this bacterial consortium was established in liquid batch cultures for three initial concentrations of MEK: 1, 2 and 3 g/l. The growth and degradation profiles were fitted by sigmoidal and logarithmic equations respectively (Figure 2) to determine growth and kinetic parameters (Table 1). Growth was fitted to a sigmoidal curve according to the following equation (Causton 1977):

$$X = m_1/[1 + exp(-m_2 + m_3.t)] + m_4 \qquad (5)$$

where X is the biomass in $g_{DCW}$/l, t is the time in hours, and the parameters had the following fitted values: $m_1$ = -1.3 $g_{DCW}$/l; $m_2$= 12.0 (non-dimensional); $m_3$= 0.32 $h^{-1}$and $m_4$= 1.5 $g_{DCW}$/l. Degradation was fitted to a logarithmic curve according to the following equation (Alexander 1994):

$$S = n_1 + n_2.(1 - exp(n_3.t)) \qquad (6)$$

where S is the residual MEK concentration in the liquid phase in g/l, t is the time in hours, and the parameters had the following fitted values: $n_1$ = 2.7 g/l; $n_2$= 0.026 g/l and $n_3$= 0.11 $h^{-1}$. Moreover, the measurement of $CO_2$ and $O_2$ in the closed flasks confirmed that MEK was totally mineralized according to Eq. 4. Only the biomass yield ($Y_{X/MEK}$) was smaller by 39% than the theoretical value (0.43 instead of 0.70 $g_{DCW}/g_{MEK}$). At all three MEK concentrations tested, the values of the kinetic parameters were of the same order of magnitude and no inhibition of MEK degradation by the pollutant itself was observed. This finding is in contrast with the work of Deshusses et al. (1996) who reported MEK self-inhibition at liquid concentrations above 0.36 g/l for a mixed microbial culture degrading this compound in batch culture. The value of the maximal specific growth rate (0.16 $h^{-1}$) was the same as that measured in the case of methanol by Shareefdeen et al. (1993) for a bacterial consortium in shake-flask experiments.

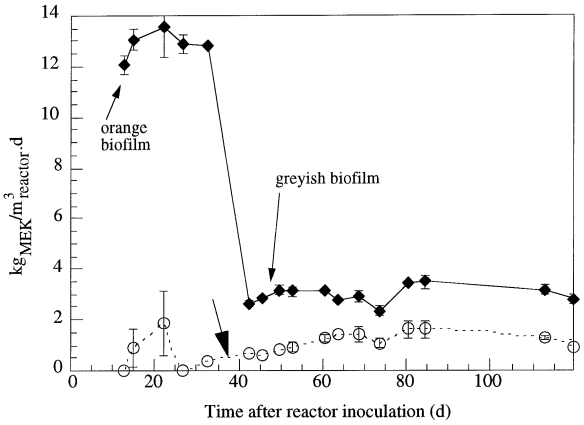


*Figure 3.* Time course of the volumetric MEK biodegradation rate ($r_v$,○) and the corresponding applied volumetric load ($B_v$, ◆) after inoculation of the DTB bioreactor with the original acclimatized bacterial consortium. The vertical bars represent the standard deviation of the state values and are calculated from at least three measurements of each term in Eq. 1. The arrow indicates the point in time when the influent volumetric load $B_v$ was reduced in an effort to stimulate the establishment of a biofilm with higher biodegradation capacity. The experimental conditions were as follows: $Q_{v,1}$ = 0.632 $m^3_l/m^3_{reactor}$·d and $Q_{v,g}$ = 1,263 $m^3_g/m^3_{reactor}$·d.

In other batch experiments with successive MEK injections of 3 g/l inside the original vessels without renewing the medium (only oxygen was added when depleted), further MEK degradation caused medium acidification (results not shown). Bacterial growth and MEK degradation stopped when pH decreased to 5.5. However, no specific acidic metabolites (acetic, formic acid, etc.) were detected under these conditions. The suspicion that this acidification was due to the generation of protons from the consumption of ammonium as a source of nitrogen was confirmed in later experiments, when the ammonium sulfate was replaced by potassium nitrate (see below).

### *Implementation of the original bacterial consortium in the bioreactor DTB*

The DTB bioreactor was inoculated by completely filling the tube with a 19-l bacterial liquid culture and by aerating it in the up-flow mode. Nineteen grams of MEK were initially added to the liquid culture. Each time MEK was completely consumed, the same initial amount of MEK was introduced again. After one week, the bioreactor was emptied. Immediately afterwards, the liquid atomization and the polluted gas flow were started.

In a first step, the MEK load was about 12 $kg/m^3_{reactor}$·d to exert a selective pressure on the microorganisms and avoid fungal contamination (Figure 3). Under these conditions no degradation could be measured, but an orange, smooth and stable biofilm formed on the glass walls. To allow the expected degradation to occur, the MEK load was decreased to about 3 $kg/m^3_{reactor}$·d and a volumetric MEK degradation rate of 0.6 $kg/m^3_{reactor}$·d was measured. However, after the 46th day of operation, the biofilm appearance and behavior had changed, becoming greyish, more and more filamentous and it frequently detached in large fragments from the side walls. These changes were caused by the evolution of the microbial composition of the biofilm due to the non-axenic experimental conditions. The greyish biofilm was more efficient than the orange one and was able to degrade up to 1.5 kg $MEK/m^3_{reactor}$·d.

The microbial composition, densities and characteristics of this new biofilm were established on the top and the body of the bioreactor, as shown in Table 2. This distinction between these two parts of the bioreactor was due to the difference in the biofilm external morphology. The biofilm in the upper part of the bioreactor (bioreactor top) was thinner, smoother and less filamentous than the biofilm in the lower part, probably because of the proximity of the atomizer orifice. Indeed, due to its Venturi effect, the atomizer could cause erosion and shear stress by projection of droplets directly on the surface of the biofilm. At this height in the reactor, the mist was very turbulent. This could prevent filamentation of fungi and biofilm growth in thickness. On the contrary, the biofilm fungal density was greater in the lower part of the biofilm (bioreactor body).

The humidity of the greyish biofilm in the upper part of the reactor was higher than in the lower part. This was also due to the direct contact of the atomized liquid droplets with the upper biofilm whereas the lower biofilm received only some droplets from the stagnant mist present at this height in the reactor. Consequently, a zone of abrupt pH transition was found to exist between the two types of biofilm. The acidic conditions in the lower biofilm may have promoted fungal development in this part of the bioreactor (De Hoog et al. 1986). A sample of this biofilm was suspended and diluted in 0.9% saline and subsequently spread on DYPA plates. Upon growth, equal proportions of two fungi were obtained. They have been identified as *Geotrichum candidum* and *Fusarium oxysporum*. These filamentous molds are known to colonize biofilms in biotrickling filters and wastewater treatment sludges and to cause clogging problems (Anderson 1983; Meyers 1984). A yeast strain was also

*Table 2.* Microbial densities and other characteristics of the MEK-degrading biofilm on the upper and lower part of the reactor

| Biofilm characteristics | Bioreactor top (wall surface area = 1854 $cm^2$) | Biorector body (wall surface area = 1854 $cm^2$) |
|---|---|---|
| Fungal density on DYPA plates containing antibacterial antibiotics | $3.10^5$ CFU/g dry weight | $64.10^5$ CFU/g dry weight |
| Yeast density on DYPA plates containing antibacterial antibiotics | $4.10^3$ CFU/g dry weight | $21.10^5$ CFU/g dry weight |
| Bacterial density on PCA plates containing fungicides | $79.10^5$ CFU/g dry weight | $54.10^5$ CFU/g dry weight |
| Total dry weight | 11 g | 50 g |
| Dry weight/wet weight | 0.04 g/g | 0.08 g/g |
| Dry weight per unit of bioreactor wall surface area | 0.006 g/$cm^2$ | 0.027 g/$cm^2$ |
| Mean thickness | 1.5 mm | 3.4 mm |
| pH on biofilm surface | 7.1 – 7.3 | 7.1 to 3.5 from top to bottom (mostly acidic) |

present on the DYPA plates in a smaller proportion than the two fungi. The bacterial part of the biofilm was always constituted of *Alcaligenes denitrificans*. The proportion of this strain (30% of the total number of bacterial colonies counted on PCA medium) was smaller than in the original consortium used to inoculate the DTB bioreactor because of the contamination by other bacterial strains.

*Favorable effect of the presence of non-bacterial microorganisms in the biofilm*

In order to determine the ability of the non-bacterial part of the active biofilm to degrade MEK, a suspension of this biofilm was inoculated in liquid batch cultures in the presence of antibacterial antibiotics (chloramphenicol and tetracycline HCl, 0.3 g/l each). Under these conditions, MEK was degraded as rapidly as without antibiotics and was completely consumed in the absence of bacterial growth (as confirmed by the absence of bacterial colonies on PCA medium). Moreover, in spite of successive MEK injections into the flask and the resulting acidification of the medium (pH dropped to 2), the degradation still continued, whereas bacterial activity was inhibited at a pH of 5.5 when the original consortium was used. The fungi had an activity of the order of 0.35-0.4 g MEK/g DCW. h. This is close to the maximum obtained at pH 7 with the bacterio-fungal consortium, i.e., 0.5 g MEK/g DCW. h.

To confirm the positive effect of non-bacterial microorganisms in MEK degradation, isolated *Geotrichum candidum* and *Fusarium oxysporum* from the biofilm were cultivated separately in liquid batch cultures. The two fungal strains were able to degrade MEK (3 g/l) completely within two days (results not shown). On the contrary, the isolated yeast strain was unable to degrade MEK. Consequently, the fungal contamination of the biofilm could be favorable in the bioreactor process, especially in the most acidic parts of the biofilm where bacteria are suspected to be partially or totally inactive. However, it must be kept in mind that *Fusarium oxysporum* is able to produce antibacterial compounds, as reported by Drysdale (1984) and Moss (1984).

*Biofilm behavior on the bioreactor walls*

As previously mentioned, the greyish biofilm was unstable on the bioreactor walls. This means that biofilm detachment occurred by the gliding of large fragments leaving no apparent biofilm on the glass surface. The possible causes of this sloughing are numerous: shear stress, pH or osmotic changes, weakening of the basal biofilm layer due to the lack of oxygen and/or MEK transported by diffusion, etc. (Characklis 1990; Applegate & Bryers 1991). In this case, it seems that the excess weight of the biofilm initiates its detachment. The sloughing is probably due to the existence of an internal network of branched fungal hyphae which joins together all parts of the biofilm covering the bioreactor walls and may drag them downwards when a part of the biofilm is detached.

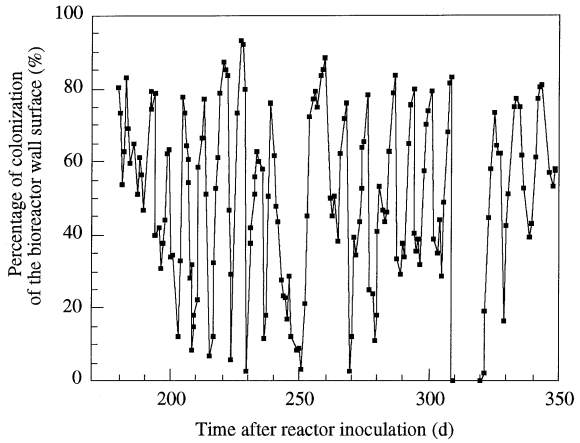


*Figure 4.* Time course of the visually estimated percentage of the bioreactor wall surface colonized by the active biofilm between the 180th and the 350th days of operation. The following experimental conditions were used: $B_{v,MEK,in}$ = 3 kg/m$^3_{reactor}$·d, $Q_{v,1}$ = 0.632 m$^3_l$/m$^3_{reactor}$·d and $Q_{v,g}$ = 1,263 m$^3_g$/m$^3_{reactor}$·d.

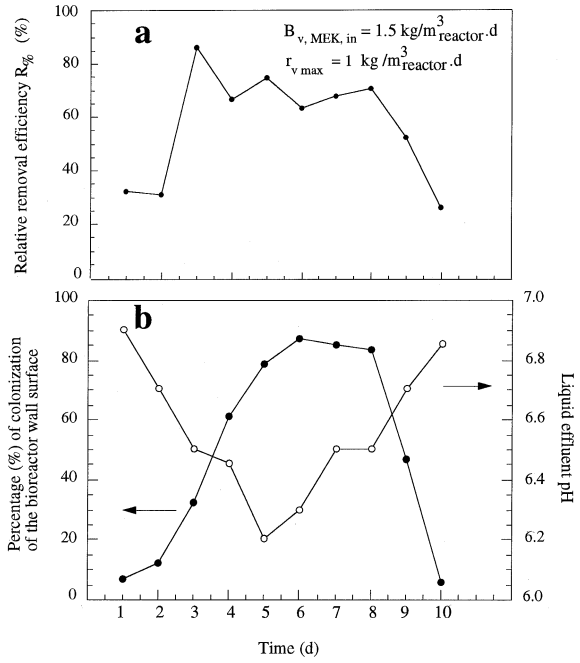


*Figure 5.* Dynamics of biofilm colonization and MEK removal over a cycle or period of time computed from the pint of complete prior detachment of the biofilm. (a) Time course of the MEK relative removal efficiency for an influent MEK load of 1.5 kg/m$^3_{reactor}$·d. (b) Time course of the percentage of the bioreactor wall surface covered by visible biofilm (●) and of the pH of the liquid effluent (○) during a cycle of biofilm detachment-recolonization in the DTB bioreactor. The following experimental conditions were used: $Q_{v,1}$ = 0.632 m$^3_l$/m$^3_{reactor}$·d and $Q_{v,g}$ = 1,263 m$^3_g$/m$^3_{reactor}$·d.

Figure 4 shows the time course of the visually estimated colonization of the bioreactor wall surface. It can be pointed out from this figure that a reproducible detachment-recolonization cycle exists in the bioreactor. This cycle lasts six to nine days. During this period, about 60 g of biofilm dry weight is produced for an influent MEK load of 3 kg/m$^3_{reactor}$·d. After a substantial period of observation in excess of 350 d, during which the reactor was run under constant conditions, it was decided to determine the relationship between the degradation performance and the state of colonization of the reactor at different applied organic loadings. Mass balances were carried out for three influent MEK loads (1.5, 3.5 and 5.5 kg/m$^3_{reactor}$·d) where each level was applied during a quasi-steady-state operation of the biofilm colonization/detachment cycle (Figure 5a and 5b). For these three applied MEK loads, the time course of the degradation performance was exactly the same (Figure 5a) for a characteristic inlet MEK load of 1.5 kg/m$^3_{reactor}$·d. At the beginning of the cycle, the relative removal efficiency rapidly increased but reached its maximum value when only 32% of the wall surface was colonized by visible biofilm. Upon increase of the influent MEK load to 1.5, 3.5 and 5.5 kg/m$^3_{reactor}$·d, the volumetric biodegradation rate increased (1, 1.6 and 1.9 kg/m$^3_{reactor}$·d respectively) whereas the relative removal efficiency decreased (67, 43 and 35% respectively) (data not shown). With an extending visible colonization of the bioreactor, the performance surprisingly did not increase further. At the end of the cycle, with biofilm sloughing, the biodegradation capacity decreased and concomitant increase of the liquid effluent pH was observed (Figure 5b). The pattern of variation in biodegradation capacity as a function of surface colonization was essentially the same irrespective of influent MEK load applied (data not shown).

The increase of the volumetric degradation rate with the inlet MEK load indicates that the reactor was possibly limited by the mass transfer of MEK with half-order kinetics (Harremoes 1978; Harremoes & Henze 1996). This increase may mean that the reactor entered progressively a biologically-controlled regime (LaMotta 1976; Andrews & Noah 1995) or that the degradation was increasingly affected by the local pH conditions or by the production of unknown metabolites. It could be argued that the local pH conditions might become worse with the increasing MEK load, as seen from the drastic pH gradient measured vertically on the biofilm surface in the lower part of the reactor (see Table 2) and from the acidification of the liquid effluent during the cycle (Figure 5b). From this, it is evident that the washing of the biofilm and the buffer-

ing capacity of the atomized liquid were insufficient to control the pH of the lower biofilm under the conditions employed. A similar situation was encountered by Kirchner et al. (1996) who showed that at high propionaldehyde loads propionic acid accumulates causing the decrease of the reactor performance. On the other hand, MEK self-inhibition should be excluded because the maximum MEK concentration measured in the bioreactor effluent liquid was too low to prevent growth as shown in batch cultures. Indeed, the MEK concentration in the bioreactor effluent liquid increased to 1 g/l for an influent MEK load of 5.5 kg/m$^3{}_{reactor}$·d. Finally, the fact that the volumetric MEK degradation rate did not increase with the colonization surface could be explained by the existence of a very thin biofilm with maximum activity, i.e. a biofilm whose thickness would be limited to a quasi invisible biomass layer (a few micrometers). The existence of a critical thickness above which substrate removal is no longer dependent on biofilm thickness has also been observed by LaMotta (1976). On the other hand, a film thicker than a few micrometers is required for complete MEK consumption (Harremoes & Henze, 1996). The pH increase of the effluent (Figure 5b) does not coincide with the sloughing of the biofilm but rather precedes it, also for the other loads (3.5 and 5.5 kg/m$^3{}_{reactor}$·d) tested. This implies that total cell activity and viability may have been damaged by a factor associated with the MEK degradation process, while cellular enzymatic activity could still be maintained until physical loss of the biofilm matrix. The ammonium consumption-driven generation of protons that would lead to an acidification concomitant with MEK degradation and growth arrest may also be at the root of these observations, if it is recognized that effluent pH is slightly out of phase with the 'average' pH in the biofilm interior (the pH varies with location, as a function of the distance from the top of the reactor).

*Effect of MEK load on removal rate*

Figure 6 shows the relation between the volumetric MEK degradation rate and the volumetric MEK load on the active biofilm. At low MEK loads, the relative removal efficiency decreased rapidly from its maximum measured value (70% for a volumetric MEK load of 0.75 kg/m$^3{}_{reactor}$·d), as already observed during the detachment-recolonization cycle. The maximum volumetric MEK removal rate was about 3 kg/m$^3{}_{reactor}$·d. This maximum value has also been obtained by Deshusses et al. (1995), but the MEK removal was complete in their biofilter. This difference in relative elimination efficiency could be due to problems of MEK mass transfer linked to the restricted exchange surface in the DTB bioreactor, to the lack of sufficient affinity of our microorganisms for MEK or to adverse environmental conditions inside the biofilm. These conditions could become worse with increasing MEK loads. The first hypothesis is the most plausible. Indeed, several authors have shown that the shorter the gas residence time, the smaller the pollutant load above which the relative removal efficiency falls below 100% (Kirchner et al. 1987; Deshusses & Hamer 1993; Deshusses et al. 1995). Therefore, a lack of sufficient gas contact time could be responsible for the profile of the degradation curve obtained here. However, at any MEK load, the ratio between the effluent gas and liquid concentrations was close to the Henry constant $5.10^{-3}$, suggesting that the overall removal rate is entirely governed by the biological reaction. At very high MEK loads, no degradation was measured. At these loads, the MEK concentration in the liquid phase was approximatively 3 g/l (for an inlet MEK load of 15 kg/m$^3{}_{reactor}$·d). Under such conditions, the biofilm was probably overloaded and poisoned by acidic metabolites or by acidification from a mineral acid (see below). The hypothesis of acid metabolite generation has already been confirmed in the case of an ethanol-removing biofilter (Devinny & Hodge 1995). However, although the conditions are different, no problems have been encountered in this range of MEK concentration for liquid batch cultures.

The decreased removal efficiency at a higher range of substrate loadings are consistent with half-order kinetics (Harremoes 1978; Janning et al. 1995; Harremoes & Henze 1996).

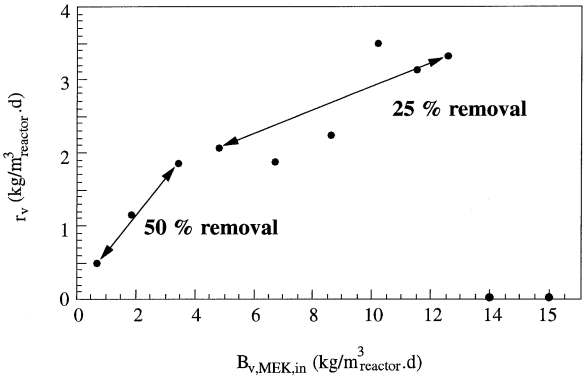


*Figure 6.* Volumetric degradation rate versus volumetric influent MEK load for the competent biofilm in the DTB bioreactor at $Q_{v,1}$ = 0.632 m$^3_l$/m$^3_{reactor}$·d and $Q_{v,g}$ = 1,263 m$^3_g$/m$^3_{reactor}$·d.

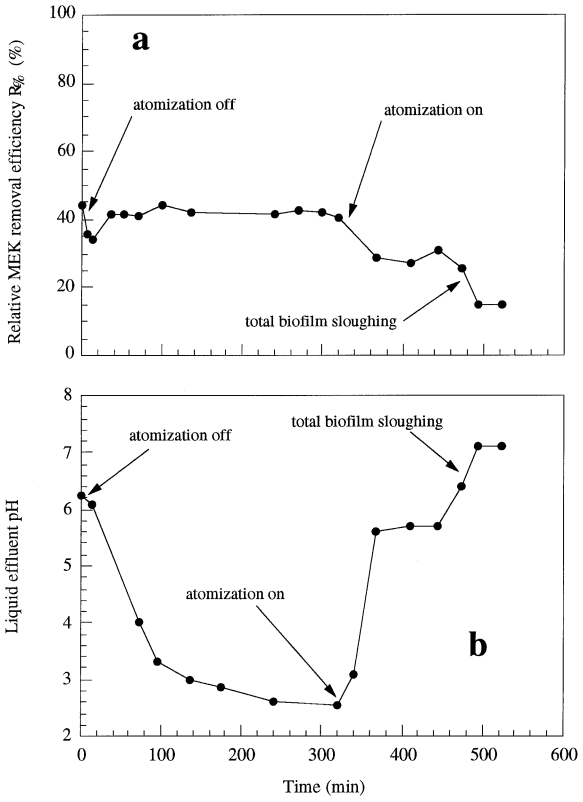


*Figure 7.* Time course of (a) the relative MEK removal efficiency and of (b) the pH of the liquid effluent after stopping the atomization of the liquid medium (at t = 0) for a $B_{v,MEK,in}$ = 5.5 kg/$m^3_{reactor}$·d, $Q_{v,1}$ = 0.632 $m^3_l/m^3_{reactor}$·d (before stopping) and $Q_{v,g}$ = 1,263 $m^3_g/m^3_{reactor}$·d.

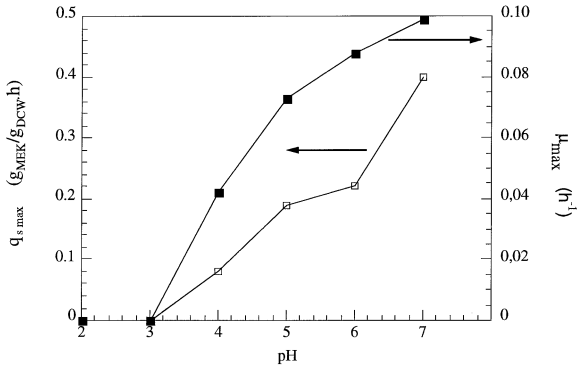


*Figure 8.* Maximum specific degradation rate ($q_{smax}$, □) and maximum specific growth rate ($\mu_{max}$, ■) versus the pH of the culture medium for microorganisms taken from the active biofilm and cultivated in closed liquid batch cultures. The initial MEK concentration was 3 g/l and the concentration of the inoculum was 0.25 $g_{DCW}$/l.

### *Factors limiting MEK degradation performance in the DTB bioreactor*

Three experiments were carried out to explore some of the factors limiting MEK degradation in the DTB bioreactor. These experiments were done after 350 days, when the dynamics of the reproducible cycles of biofilm colonization and detachment had been well established.

#### *Effect of the presence of the mist*

The wetting of the biofilm in trickling filters could affect the overall degradation rate (De Heyder et al. 1994). To determine the probable negative (mass transfer resistance) or positive (biofilm washing, pH control, nutrient supply) effects of the liquid on the reactor performance, the atomization of the liquid medium was temporarily stopped, but the gas flow rate and the influent MEK load (5.5 kg MEK/$m^3_{reactor}$·d) were kept constant (Figure 7a and 7b). Over a period of five hours, the biofilm was being depleted of water. Mass balances were carried out before and during the drying period. The degradation performance ($r_v$ = 2.3 kg/$m^3_{reactor}$·d) remained the same (Figure 7a). The MEK removal rate decreased only when the liquid was atomized again ($r_v$ = 1.5 kg/$m^3_{reactor}$·d). Finally, the biofilm sloughed off as a single block during its re-humidification. The liquid collected at the exit of the reactor was rapidly becoming more and more acidic during the dry air period and the pH reached a minimum of 2.5 (Figure 7b). No specific acidic metabolites were found either in this liquid, or in the biofilm. This suggested that a mineral acid is involved (production of protons) rather than an organic acid, since the pKa's of organic acids are too high to allow a pH drop down to such a low level as 2.5. Acetic acid, a potential metabolite, as proposed in the possible metabolic pathways of the degradation of acetone (Taylor et al. 1980) and ketone-transformed n-alkanes (Rittmann et al. 1994), was not detected in these samples. Evidence consistent with a mechanism of proton production associated with the utilisation of ammonium in the course of MEK metabolism is given below in a set of final experiments.

Since the biodegradation performance was not enhanced, it can be concluded that, in the presence of the mist, no limitation due to the mass transfer of MEK from the gas to the biofilm exists in the bioreactor. This is probably due to the small size of the droplets which favors rapid gas/liquid exchanges and generates a very thin water layer on the biofilm. The cause of the limitation of the degradation capacity in the reactor must be biological. The degradation performance was surprisingly maintained when biofilm wetting was temporarily stopped. This could be attributed to the

degradation capacity and the relative tolerance of fungal microorganisms towards biofilm acidification, as shown in batch experiments, and to the possible storage of nutrients inside the biofilm, but further research is warranted on this point.

*Effect of oxygen diffusion inside the biofilm*

It is well-known that oxygen diffusion inside the biofilm is sometimes rate-determining because of its poor solubility (Kirchner et al. 1996). To test this possibility, an experiment was conducted during a one-day quasi-steady state interval of operation within a single expansion/detachment cycle (approximately in its latter one-third portion, when substantial recolonisation had occurred). The oxygen concentration in the air was increased to 77% (v/v) when the biofilm appeared visually thick. This would increase the oxygen penetration depth and the active biofilm thickness. The degradation performance did not improve (data not shown). Therefore, oxygen diffusion within the biofilm did not seem to be rate-limiting, even for high MEK loads.

*Biofilm pH limitation*

To determine quantitatively the pH effect on biodegradation activity and growth of microorganisms, a homogeneous suspension of a biofilm sample taken from the reactor was used to inoculate batch cultures at six different pH values, as shown in Figure 8. The optimal pH was around 7. In another experiment carried out with microorganisms isolated from the biofilm on solid media, it was shown that the artificially reconstituted bacterial consortium was only able to degrade MEK at pH 6 and 7, *Geotrichum candidum* alone at pH 5, 6 and 7, and *Fusarium oxysporum* alone at pH 4, 5, 6 and 7 (data not shown).

On the basis of these results, the pH of the atomized liquid was adjusted to 6.7 with a very well buffered medium ($Na_2HPO_4.2H_2O$ 5.34 g/l, $KH_2PO_4$4.08 g/l) for a better control of the biofilm pH. Mass balances were carried out for different MEK loads. Under these conditions, the pH everywhere on the biofilm surface was kept quite close to the pH of the atomized liquid. The performance of the bioreactor improved. A maximum volumetric MEK degradation rate of 4.8 kg/$m^3_{reactor}$·d was obtained for a MEK load of 12 and 18 kg/$m^3_{reactor}$·d (data not shown). This is consistent with the behavior observed in analogous systems (Weckhuysen et al. 1993). Even though no significant increase of the removal yield was obtained, the value of 4.8 kg/$m^3_{reactor}$·d still represents a maximum removal rate of MEK at such high volumetric loads, which is clearly a consequence of biofilm pH optimization.

In a final series of experiments designed to obtain confirmatory evidence that the acidification was likely due to the accumulation of protons from a mineral acid, batch incubations were carried out in flasks containing 0.25 g DCW/l of biofilm microbial mass in a medium where the ammonium sulfate (9 g/l) was replaced by potassium nitrate (14 g/l). The medium of incubation was kept identical with the one described above (see Materials and Methods) except for an amendment with sodium sulfate (9.68 g/l) designed to give an equivalent content in sulfate with the ammonium-containing control. In parallel batch incubations, after 48 h, 10g/l of MEK had disappeared and the pH had remained stable at 7 for the nitrate-containing flasks, whereas for the ammonium-containing flasks 5 g/l of residual MEK remained in the liquid and the pH had fallen to 5.5. Other data indicated that the degradation of only 3g/l of MEK by the mixed population of the biofilm provoked an acidification of the medium by 1.5 pH units within 20 h and this acidification blocked completely any further MEK degradation (results not shown). In an analogous experiment but with biomass constituted from the two fungal species of the biofilm there was full degradation of 10 g/l MEK within 72 h and a stable pH of 7 in the nitrate-containing medium, whereas the degradation of the same concentration of MEK was possible even in the presence of medium acidification, yet completed only after 240 h (results not shown).

## Conclusion

Despite the regular periodic detachment of the biofilm and a tendency of the biofilm towards acidification, which affected adversely the bioreactor performance, a comparison with results for MEK-removing bioreactors in the literature (Kirchner et al. 1987; Deshusses & Hamer 1993; Deshusses et al. 1995) shows that the maximum removal rate (4.8 kg MEK/$m^3_{reactor}$·d) measured in the DTB bioreactor is very promising. To obtain a better performance in relative removal efficiency, care will have to be taken of the following:

- The surface for the biofilm development should be increased at the same biofilm thickness (1.5-3.0 mm). To maintain a thin biofilm along the reactor wall, the shear stress caused by the atomization or the effect of the drying could be reduced by using multiple nozzles and/or examining the effects of their location in the reactor.
- The retention of microorganisms inside the reactor must be improved, possibly by modifying the wall surface.

- The acidification of the biofilm must be controlled by a non proton-generating nitrogen source (nitrate) and judicious phosphate buffering or neutralization with calcium carbonate, periodic washing with spurts of water, etc. (Smet et al. 1996).

## Nomenclature

| | | |
|---|---|---|
| $B_{v,g,in}$ | $kg_{MEK}/m^3_{reactor}\cdot d$ | volumetric MEK load in influent gas |
| $B_{v,g,out}$ | $kg_{MEK}/m^3_{reactor}\cdot d$ | volumetric MEK load in effluent gas |
| $B_{v,l,out}$ | $kg_{MEK}/m^3_{reactor}\cdot d$ | volumetric MEK load in effluent liquid |
| H | – | Henry's constant |
| $[MEK]_{g,in}$ | $kg/m^3_g$ | influent MEK gas concentration |
| $[MEK]_{g,out}$ | $kg/m^3_g$ | effluent MEK gas concentration |
| $[MEK]_{l,out}$ | $kg/m^3_l$ | effluent MEK liquid concentration |
| $\mu_{max}$ | $h^{-1}$ | maximum biomass specific growth rate |
| $q_{smax}$ | $g_{MEK}/g_{DCW}.h$ | maximum specific degradation rate |
| $Q_{v,g,in}$ | $m^3_g/m^3_{reactor}\cdot d$ | volumetric influent gas flow rate |
| $Q_{v,g,out}$ | $m^3_g/m^3_{reactor}\cdot d$ | volumetric effluent gas flow rate |
| $Q_{v,l,out}$ | $m^3_l/m^3_{reactor}\cdot d$ | volumetric effluent liquid flow rate |
| $r_v$ | $kg_{MEK}/m^3_{reactor}\cdot d$ | volumetric MEK removal rate |
| $R_{\%}$ | % | percentage of removal or relative MEK elimination efficiency |
| S | g/l | substrate (MEK) concentration in liquid batch |
| X | $g_{DCW}$/l | biomass concentration in liquid batch |
| $Y_{X/MEK}$ | $g_{DCW}$/g | yield of conversion of MEK into biomass |
| $Y_{O2/MEK}$ | mole/mole | yield of oxygen consumption related to MEK degradation |
| $Y_{CO2/MEK}$ | mole/mole | yield of carbon dioxide production related to MEK degradation |
| $Y_{N/MEK}$ | mole/mole | yield of nitrogen consumption related to MEK degradation |
| $Y_{X/O2}$ | $g_{DCW}$/g | yield of biomass production related to oxygen consumption |
| $Y_{X/CO2}$ | $g_{DCW}$/g | yield of biomass production related to carbon dioxide production |
| $Y_{X/N}$ | $g_{DCW}$/g | yield of biomass production related to nitrogen consumption |

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