Model description and kinetic parameter analysis of MTBE biodegradation in a packed bed reactor

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1. Introduction

Due to its technical properties as well as a low cost and ease of production, methyl tert-butyl ether (MTBE) has been used worldwide since the 1970s as a fuel oxygenate added to gasoline. Although it helps to improve air quality, it often creates serious drinking water problems due to its low odor and taste threshold of around 2–2.5 mg/L (Fiorenza and Rifai, 2003). MTBE has contaminated many water supplies worldwide (Morgenroth and Arvin, 2003; Stocking et al., 2000); more than 400,000 leaking underground storage tanks sites in the US has been identified since 1988 (Small et al., 2002). Although MTBE is being phased out in many US states it will continue to be a groundwater problem in the future (Johnson et al., 2000), mainly due to the very slow attenuation under natural groundwater conditions (Landmeyer et al., 1998).

Common physicochemical treatment technologies for MTBE are, e.g., air stripping, soil vapor extraction, adsorption on activated carbon, and advanced oxidation techniques using O3/H2O2, UV/H2O2 or Fenton’s reagent. Selection of the technology used for treatment of MTBE will, however, have to be done on a case-by-case basis and may include factors such as cost effectiveness, operability and drinking water guidelines (Melin, 2000; Sutherland et al., 2004). This article, however, focuses on the use of bioremediation as a treatment option.

Fixed biofilm reactor systems are effective for bioremediation of MTBE polluted groundwater (Kharoune et al., 2001; Prandi et al., 2002). MTBE as well as other groundwater co-contaminants such as ammonium, benzene, toluene, ethylbenzene and xylenes can be present together at contaminated sites (Martienssen et al., 2006). Therefore, the application of a single biofilm reactor for remediation of...
MTBE together with co-contaminant compounds is very desirable from an economic standpoint.

Biofilm systems have the ability to attain high biomass concentrations, and the biofilms can be immobilized thus preventing loss from the reactor. Several types of organisms can grow together independent of their growth rates, and they are generally robust in relation to toxicity effects from chemicals (Characklis and Marshall, 1989; Henze et al., 1997).

To predict reactor performances, conduct designs and the model systems, obtaining reliable growth kinetic parameters of the involved organisms is very important. Parameter estimation of the maximum growth rate ($m_{\text{max}}$) and the half-saturation constants ($K_S$) are pivotal for the description of substrate removal and biomass growth in a microbial system, which is modeled with Monod kinetics. There are techniques discussed in the literature for estimating the parameters in suspended culture systems; however, there are no fast and accurate techniques for biofilm systems (Riefler et al., 1998). It may be possible to remove biofilm samples and perform batch tests; however, the results of such tests may not represent the intrinsic kinetics of the system (Grady et al., 1996). Conducting parameter estimates under in-situ conditions with an already operating biofilm reactor is best for obtaining parameters, which are representative of the system concerned.

Experiments conducted such that the substrate concentration varies well below and above the $K_S$ value will improve the uniqueness of the model parameters and can be achieved through dynamic modeling. A pulse of substrate is injected to a reactor’s inlet and the magnitude of the response in the substrate concentration vs. time is measured at the outlet (Dochain and Vanrolleghem, 2001).

In this study, a mathematical model that can be used to study the simultaneous oxidation of MTBE and ammonium in a plug flow packed bed reactor (PBR) has been developed. The model parameters are estimated under dynamic modeling conditions by injecting pulses of MTBE and/or ammonium to the reactor's inlet. Least-squared analysis and a response surface methodology was used to locate the optimal model parameters. It is intended that the model and parameter estimates found may be useful for design or optimization of similar MTBE-degrading systems.

2. Materials and methods

2.1. Reactor setup and operation

A PBR operated as a plug flow system with dimensions of 0.11 m diameter and 1 m depth was used as the experimental system (Fig. 1). The objective for building the reactor was to obtain MTBE degradability information, which could be used for construction of bioremoval systems at the MTBE polluted site in Leuna, Germany. High MTBE and ammonium concentrations (> 100 ppm) were measured in the groundwater at this site (Martienssen et al., 2006). When the reactor was first started up, it was operated for about 2 years where the temperature, pH and oxygen dependency including different methods of supplying oxygen for degradation of MTBE were assessed (Arvin and Krag, 2002). The operating conditions during the startup period were not always the same as those described in the following section since different reactor conditions were tested.

The MTBE-degrading cultures were supplied by Ulrick Karlson from the National Environmental Research Institute (NERI), Denmark and were obtained from the Leuna site in Germany (Martienssen et al., 2006).

Table 1 shows the inlet conditions of the reactor within the last year prior to the start of the dynamic experiments. The feed stock solution to the reactor consisted of MTBE, (NH₄)₂SO₄ and KH₂PO₄ in distilled water. This stock solution was diluted online with local tap water from Lyngby near Copenhagen, Denmark. Pure oxygen was supplied to the reactor.
system using a stone diffuser placed in the tap water storage tank (Fig. 1).

2.2. Analytical methods and chemicals

Nitrate and sulfate analysis was conducted using Spectroquant® measuring kits (Merck, Germany) and a spectrophotometer (Spectroquant® NOVA 60). Ammonium was measured by a colorimetric method using an auto analyzer (Autoanalyser 3; Bran+Luebbe) according to standard methods (APHA, 2005). The dissolved oxygen concentration was measured with a Unisense® Oxi probe or Clark-type electrodes (WTW, Germany). Conductivity measurements were done using a conductivity-measuring instrument (WTW, Germany).

MTBE and tert-butyl ether (TBA) were measured using the Purge and Trap method with a Tekmar, LSC 2000 instrument coupled to a Shimadzu GC 14B instrument with flame ionization detector according to US EPA method 5030C (EPA, 2003). The gas chromatograph (GC) was set at an initial oven temperature of 40°C, ramped at 10°C/min to 140°C. The detector was set at 340°C, and nitrogen was used as the carrier gas, set at 50 kPa. The GC was fitted with an Agilent Technologies HP-5 column of length 50 m, internal diameter 0.2 mm and film thickness of 0.11 μm. Samples were normally stored previously to analysis in 25 mL purge and trap glass vials at −18°C. Vials were capped with plastic lids containing Teflon-lined silicone septa and had only a small headspace for expansion when frozen.

Adenosine triphosphate (ATP) measurements were done using a luminometer (Advance coupe, Celsis, Landgraaf, The Netherlands). The method has been described elsewhere (Silhan et al., 2006). Chemicals of the highest purity available were purchased from Merck (Darmstadt, Germany).

2.3. Determination of a preliminary estimate of $K_S$

A preliminary determination of $K_S$ was done as an independent experiment using the setup shown in Fig. 1. The inlet MTBE concentration of the PBR was varied from 4.1 to 35.5 g COD/m³ and the oxygen concentrations kept close to 40 g/m³, just for the duration of these experiments. Eight new steady-state removal rates were calculated. No ammonium entered the reactor at this time and all other operating conditions were as shown in Table 1. MTBE removal rates were calculated using the difference in the concentrations entering and leaving the first 0.2 m depth of the reactor divided by the pore water retention time in that section. An estimate for $K_S$ was obtained by using the curve fitting toolbox in MATLAB. A Monod expression was used to fit the removal rate data obtained to the mean MTBE reactor concentration within the first 0.2 m depth.

2.4. Reactor pulsing and step change

A 10 mL pulse of ammonium sulfate and/or MTBE was injected at the inlet of the reactor using a syringe. The MTBE concentration at the inlet of the reactor was reduced from the normal operating value of 27.3–5.46 g COD/m³, a few hours prior to pulsing in order to record measurements over a wide range. All other substrate concentrations and hydraulic conditions were unchanged. Three separate pulses of 10 mL were conducted: (i) 4.24 kg N/m³ only; (ii) 10.16 kg COD/m³ MTBE together with 4.24 kg N/m³ and (iii) 10.16 kg COD/m³ MTBE only. Steady-state substrate conditions were ensured at the reactor’s outlet by observation of a constant oxygen concentration on an electrode connected there, prior to each of the pulses. No depletion of alkalinity occurred inside the reactor since there were minimal fluctuations of pH observed during the ammonium pulsing.

For further validation of the estimated model parameters an instantaneous step change in the inlet MTBE concentration from 5.46 to 27.3 g COD/m³ was also done. Steady-state conditions were also ensured by measuring oxygen concentrations in the reactor’s outlet prior to this. Furthermore, all other substrate concentrations and hydraulic conditions were kept at the normal operating conditions. Prior to the start of the dynamic experiments, the MTBE, nitrogen and oxygen profiles vs. depth were all at steady state within the last month.

2.5. ATP measurements

ATP measurements were done to compare the biomass activity of the reactor with model simulations. Biomass samples were taken from the reactor’s sampling ports using a rigid sterile plastic tube of internal diameter 0.5 cm. A plastic syringe connected to the end of the tube facilitated the removal of a sample containing reactor packing material and free biomass. The ATP determination of the free biomass was done using drained samples for which the quantity of volatile suspended solids (VSS) was later determined. In a separate measurement, four individual particles from each of the four-side port of the PBR were used as the control basis for determination of the ATP profile of the attached biomass.

2.6. Kinetic model description

The model was built on the same principles used in the activated sludge model 1 (ASM1) described in Henze et al. (1987). The rate expressions of all growth processes were described using Monod’s rate equations. All bacterial species were assumed to undergo decay. The rate equation was expressed as a function of the biomass concentration and modeled as a first-order process. The stoichiometric matrix and rate expressions are shown (Tables I and II, supplementary data). The following important model extensions to ASM1 were made: (1) three active bacterial species were considered in the model: MTBE degraders ($X_{MTBE}$), ammonium oxidizers ($X_{NH4}$) and general heterotrophs ($X_{H}$); (2) growth of MTBE degraders only occurs with MTBE, accompanied by full mineralization to carbon dioxide and water using oxygen as electron acceptor; (3) the nitrogen source for growth of the MTBE degraders can either be ammonium or nitrate (nitrate is used only if ammonium is not present); and (4) either ammonium or nitrate can be used as the nitrogen source for heterotrophic growth.

2.6.1. Justification of kinetic model extensions

Based on the current experimental evidence, there is oxidation of MTBE, nitrification and denitrification in the reactor.
Degradation of MTBE by direct metabolism is only known to occur with a few specialized organisms (Fayolle et al., 2001). Therefore, three different bacterial species were included in the model.

Despite that a few authors have reported otherwise (Finneran and Lovley, 2001; Pruden et al., 2005), degradation of MTBE under anaerobic conditions is still an extremely slow process and occurs rarely (Schmidt et al., 2004). A second similar reactor connected in series after the PBR described here showed no degradation of the parent MTBE under anaerobic conditions in over 2 years of operation. Therefore, the second model assumption is justified.

The third and forth assumptions enables biomass growth with either ammonium or nitrate. Under the normal operating conditions of the reactor, ammonium entering will become fully oxidized before all oxygen is consumed. This results in very low ammonium concentration in a part of the aerobic section and in the entire anoxic section of the reactor. Therefore, to prevent nitrogen deficiency in the MTBE and heterotrophic biomasses, nitrate must be used as the nitrogen source. This was implemented by having two separate growth processes for MTBE: one with ammonium and the other with nitrate as nitrogen source. The heterotrophic growth also occurs with ammonium under aerobic conditions and with nitrate under anoxic conditions (Tables I and II, supplementary data).

2.6.2. Kinetic model implementation
The kinetic model was implemented in the software AQUASIM 2.1 (Reichert, 1994). The saturated soil column compartment was used with dispersion and one advective zone. This compartment is specially designed for the simulation of advective-dispersive transport of dissolved components in plug flow systems. It is possible to make both time and longitudinal space simulations of the state variables. The high resolution setting with 100 grid points was used. The grid points refer to the number of equally separated points in which the longitudinal axis of the reactor is resolved (Reichert, 1994). The three active biomass fractions \( X_{\text{MTBE}} \), \( X_{\text{NH4}} \) and \( X_{\text{H}} \) as well as the inert \( X_{\text{I}} \) and hydrolysable \( X_{\text{S}} \) particulate components were implemented as dynamic surface state variables in units of gCOD/m\(^3\). Dynamic surface state variables are considered immobile within the reactor. Soluble components, for example, the inlet MTBE and ammonium were implemented as volume state variables. Diffusion aspects of a biofilm were not included explicitly in the model, however, it shown to be manifested in the \( K_s \) values (Pérez et al., 2005).

The concentration profiles of the dynamic surface state variables vs. depth in the reactor are required a priori for estimation of model parameters under dynamic conditions. These were obtained from a simulation of the model to a steady state (500 days) of the state variables using parameters mainly obtained from the literature.

The input substrate pulses to the model were implemented after 500 days of simulation time as triangles containing the total mass of the slug of MTBE or ammonium; 30 s was used as the injection time in the model. The choices of some model parameters, which are most important to the outcome of the model, have been discussed below.

2.6.3. Kinetic model parameters
The kinetic and stoichiometric parameters used in the implementation of the steady-state model prior to the estimation procedure are shown. They were in their typical ranges under the conditions of temperature and pH of the experiments (Tables III and IV, supplementary data).

The \( \mu_{\text{max}} \) value of the MTBE degraders chosen was 0.1 d\(^{-1}\), this estimate was obtained from a similar PBR reactor as described here (Arvin and Krag, 2002). The value of \( K_s \) was 17.2 gCOD/m\(^3\); this was obtained from preliminary studies using the current reactor and described earlier.

The \( K_s \) values for nitrifiers and heterotrophs in wastewater systems are 0.5–1 gN/m\(^3\) and 20 gCOD/m\(^3\) (Henze et al., 1997). The inlet ammonium concentration to the PBR is only 1, and is 0.1 gN/m\(^3\) or less at the outlet. The substrate for the heterotrophic biomass is only produced from decay; hence, the concentration inside the PBR is expected to be also very low (<1 gCOD/m\(^3\)). Furthermore, the substrate concentrations encountered by the heterotrophs and the nitrifiers in the PBR is much lower compared to values around 50 gCOD/m\(^3\) and <10 gN/m\(^3\) found inside typical wastewater treatment plants (Henze et al., 1997). For these reasons, the half-saturation constants \( K_{s,\text{NH4}} \) and \( K_{s,\text{H}} \) must be lowered to reflect these lower substrate concentrations (Choubert et al., 2005).

They were set to 0.2 gN/m\(^3\) and 0.5 gCOD/m\(^3\), respectively.

The decay constant \( b \) for MTBE degraders was set to 0.01 d\(^{-1}\). Based on values in Henze et al. (1997), decay constants are in the order of 10% of the maximum growth rates.

The initial biomass concentration, of \( X_{\text{MTBE}} \), \( X_{\text{NH4}} \) and \( X_{\text{H}} \) used to conduct the steady-state simulations were all set to a low value of 1 gCOD/m\(^3\) over the entire longitudinal profile of the reactor.

A hydraulic model of the reactor was implemented in AQUASIM 2.1 (Reichert, 1994). A profile of conductivity vs. time laboratory measurements was used to determine the dispersion coefficient, mean retention time and the porosity of the reactor (Fig. 1, supplementary data). These were found to be 6.85 m\(^2\)/d, 23 min and 0.45, respectively.

2.6.4. Biofilm attachment and detachment
Attachment and detachment of biomass at the biofilm’s surface in the PBR cannot be inherently modeled in the saturated soil column compartment, for example, by means of a global velocity (Wanner and Morgenroth, 2004). Therefore, the total active biomass concentrations \( X_{\text{MTBE}}, X_{\text{NH4}} \) and \( X_{\text{H}} \) were modeled to reach a maximum, or saturation, in order to prevent unrealistic values mainly at the reactor’s inlet, where the substrate fluxes are highest. Effectively, this is a way of reaching steady-state biomass concentrations in the reactor, which is similar to defining a maximum biofilm thickness. The maximum active biomass concentrations reached were governed by the use of a saturation function:

\[ \frac{K_s}{X_{\text{TOT}} + K_x} \]

Upon inspection of the function, it can be deduced that it approaches zero as \( X_{\text{TOT}} \) begins to exceed the value for \( K_x \). Therefore, when this function is multiplied by the growth expressions for the active biomass species in the model a
pseudo-steady-state concentrations can be reached (Table I, supplementary data). While there is no literature value available for \( K_X \) (biomass saturation constant), an initial estimate of 10 kg COD/m\(^3\) was chosen for deducing the biomass profiles prior to implementation of the substrate pulses in the model. This value is close to the expected biomass concentrations for such biofilm systems (Henze et al., 1997; Rittmann and McCarty, 2001).

2.7. Non-linear parameter estimation

The criteria used for determination of parameters which best described the model was based on the minimization of the sum of squared residuals (RSS). The objective function is as follows (Box et al., 1978):

\[
\min \sum_{i=1}^{n} (y_i - \hat{y})^2
\]

where \( n \) is the number of data points obtained from a pulse experiment, \( y_i \) and \( \hat{y} \) are the experimental and model simulated data sets. The transient concentration profiles recorded at the reactor’s outlet from pulsing with MTBE and/or ammonium were used as the basis for conducting parameter estimations.

A factorial design approach was used for determination of the optimal parameters for the model. The specific design chosen in this case is referred to as the central composite design (CCD). Briefly, the method is based on selecting 2\( x \)-values representing the possible extremities of the parameter to be estimated. The model parameters were varied simultaneously on five levels between the \( x \)-values (Table 2). The \( x \)-values were based on a preliminary search of a larger parameter space, using a 2-factorial design (Box et al., 1978; Montgomery, 2005). Thirty parameter combinations were parameter space, using a 2-factorial design (Box et al., 1978; Montgomery, 2005). Thirty parameter combinations were then simulated using AQUASIM 2.1 (Reichert, 1994). Two sets of RSS values (\( J_{\text{RSS}}^{2} \)) were computed from the second pulsing experiment using the experimentally measured data for both ammonium and MTBE. The \(-/+ x\)-values of the parameters and \( J_{\text{RSS}}^{2} \) data sets are referred to here as the CCD data set. Using the statistical program Design-Expert 7.1 (Statease\(^{\circledR}\)), two second-order response surface models (Eq. (1)) were constructed using the CCD data sets obtained. Individual CCD data sets were constructed from the MTBE and ammonium pulse data. The response surface models evaluates \( J_{\text{RSS}}^{2} \) as a function the model parameters to be estimated using a second-order polynomial (Box et al., 1978; Montgomery, 2005):

\[
y^2_{\text{pred}} = \hat{y}_0 + \sum_{j=1}^{p} \hat{b}_j x_j + \sum_{i<j=1}^{p} \hat{b}_{ij} x_i x_j + \sum_{j=1}^{p} \hat{b}_j^2 x_j^2 + \epsilon \tag{1}
\]

where \( \hat{b}_j, j = 0, 1, \ldots, p \) are the regression coefficients, \( x_i, x_j \) are the model parameters, \( \epsilon \) is the residuals and \( p \) the number of parameters in the model. The interacting terms and the second-order terms are necessary to describe the curvature in the response surface (Myers and Montgomery, 2002). The response surface models were then assessed using statistical tests to determine their appropriateness and significance for describing the CCD data sets.

2.7.1. Reliability of parameter estimates

Statistical tests performed to determine the appropriateness and significance of the response surface models were as follows: (1) normal probability plot of studentized residuals, \( e_i \), (2) predicted \( J_{\text{RSS}}^{2} \) vs. \( J_{\text{RSS}}^{2} \) and (3) \( e_i \) vs. \( J_{\text{RSS}}^{2} \) and (Box et al., 1978; Montgomery, 2005). Additional statistical tests performed included computation of the model \( p \)-values and a lack of fit test. The \( p \)-values indicate quantitatively the statistical significance of how well the response surface models describe the CCD data sets.

2.7.2. Parameter optimization and confidence region

Design-Expert determines model parameters using a graphical optimization technique based on user optimization goals and the expected parameter ranges (Table 2). The goal was to determine a global minimum for \( J_{\text{RSS}}^{2} \) in between \( x \)-values simultaneously using all four parameters (Table 2). The program uses multiple starting points of the parameters between the \( x \)-values. The \( J_{\text{RSS}}^{2} \) response surfaces of the parameter interacting regions were then examined to ensure that estimates were from a global minimum.

If model residuals (\( e_i \)) are normally distributed, the critical value \( J_{\text{crit}} \), that defines the surface of the parameter confidence region, can be defined by the \( F \)-distribution (Vanrolleghem et al., 1995):

\[
J_{\text{crit}} = J_{\text{opt}} \left( 1 + \frac{\alpha \, p}{n - p} F_{\alpha,p,n-p} \right) \tag{2}
\]

where \( n \) is the number of data points in the CCD, \( J_{\text{opt}} \) and \( p \) are the global minima and number of parameters to be optimized or estimated; \( F_{\alpha,p,n-p} \) is found from the \( F \)-distribution for \( \alpha \) and \( n - p \). With \( \alpha = 0.05 \%), reading from the \( F \)-distribution, \( F_{0.05,4,26} = 2.74 \) was used for a 95% level of significance for \( J_{\text{crit}} \). Based on the \( J_{\text{opt}} \) found, the contour line representing \( J_{\text{crit}} \) (Eq. (2)) was graphed from Design-Expert.

2.7.3. Estimation of the nitrifiers kinetic and stoichiometric parameters

Parameter estimates were also carried out for the nitrifiers in the model using the procedure described here for MTBE. The parameters included in the estimation procedure were \( \mu_{\text{max}}, K_s, Y \) and the percentage ammonium sorbed (NH\(_4\)sorb) in the reactor during pulsing. The search range of the parameters \( \mu_{\text{max}}, K_s \) and \( Y \) were based on typical nitrification parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>(-z)</th>
<th>(+z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu_{\text{max}} )</td>
<td>d(^{-1})</td>
<td>0.05</td>
<td>0.275</td>
</tr>
<tr>
<td>( K_s )</td>
<td>mg COD/L</td>
<td>1e(^{-5})</td>
<td>7.5</td>
</tr>
<tr>
<td>( Y )</td>
<td>g COD/g COD</td>
<td>0.05</td>
<td>0.275</td>
</tr>
<tr>
<td>( K_s )</td>
<td>mg COD/L</td>
<td>1000</td>
<td>14,500</td>
</tr>
</tbody>
</table>

\(-/+ z\)-Values are the extremities of the actual values used in the parameter search region.
in the literature (Henze et al., 1997). The range chosen for the \( \text{NH}_4 \text{ sorb} \) was based on the values found from a mass balance of total nitrogen species during pulsing with ammonium.

### 3. Results and discussion

#### 3.1. Dynamic response data from pulse experiments

Fig. 2(a–e) shows the experimental data and model fits to the MTBE, oxygen, ammonium and nitrate concentrations recorded at the PBR's outlet during the pulsing experiments. Model fits are based on the MTBE degraders’ model parameters estimated from the second pulse, plots (c) and (d). The nitrate and oxygen data sets from the other two substrate pulses, plots (a, b) and (c), are used for qualitative assessments. Fig. 2 shows that the peak response of the MTBE or ammonium data is approximately 20–25 min, which is consistent with the data obtained from the tracer experiments. Generally, it can be observed from the figure in plots (c) and (e) that the measured MTBE data points fit the model more closely within the first 20 min than afterwards. The peak MTBE concentration observed from the measured data points then falls below that of the simulated response when oxygen has been depleted in the pulse. This discrepancy cannot be corrected by any adjustment of the MTBE degraders’ model parameters since all the oxygen is shown to be consumed. Based on the qualitative similarity of the model fits of the model to the MTBE data in plots (c) and (e), it shows that the MTBE response of the model can be replicated under different dynamic conditions.

The measured and simulated responses of the ammonium data generally peaked at the same concentration, plots (b) and (d); however, there is an obvious lag of the measured data compared to the modeled response. This is related to the sorption of ammonium on the filtralite\(^\text{TM}\) packing material inside the reactor. Nitrate is also produced above the background concentration during the ammonium pulses (Fig. 2b and d). The nitrate peak observed from the measured data remains slightly longer than the model predicts; this is due to the delayed nitrification as a result of the ammonium that was sorbed.

The deviations between the measured and the predicted oxygen response especially after 20 min (Fig. 2a and c) can also be attributed to the sorption of ammonia. Oxygen is consumed in the experimental system a longer time than the model shows; this can be explained by the lag in the measured compared to simulated ammonium response.

#### 3.2. Oxygen response to MTBE step change

Fig. 3 shows the experimental data and the model fit to the oxygen concentration at the outlet of the PBR after an

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Fig. 2 – Experimental data and model fits to the MTBE, oxygen, ammonium and nitrate data sets recorded at the packed bed reactor's outlet during the pulsing experiments. Plots (a), (c) and (e) show the MTBE (○) and oxygen (▲) data and the corresponding model fits. Plots (b) and (d) show the ammonium (□) and the nitrate (△) data as well as the corresponding model fits. The MTBE and ammonium model fits are shown in thick lines while oxygen and nitrate are in thin lines. Profiles (a) and (b) are simultaneous measurements obtained from first pulsing, done with ammonium only; (c) and (d) from second, done both with MTBE and ammonium and (e) from the third, done with MTBE only. Data sets of the second pulsing, plots (c and d) were used to conduct parameter estimates under dynamic conditions.
instantaneous increase of the MTBE concentration at the inlet, from 5.46 to 27.3 g COD/m³. The simulations were done with the optimum parameter sets (Table 3). An observable good fit between the simulation results and experimental data provides further confidence in the model and the parameters estimated.

3.2.1. Parameter confidence regions

Fig. 4 shows the three-dimensional response surface ($\nu^2_{\text{pred}}$) plot for the MTBE degraders’ $K_S$ and $\mu_{\text{max}}$. The plots were drawn using the final optimal parameters for the MTBE degraders (Table 3). The plot shows a well-defined valley where the optimum values of $\mu_{\text{max}}$ and $K_S$ resides, which indicates good identifiability of these parameters. Generally, there is a greater sensitivity of the model to $\mu_{\text{max}}$ compared to $K_S$. There is a greater change in chi-squared ($\nu^2_{\text{pred}}$) on the $\mu_{\text{max}}$ compared to the $K_S$ axis.

Fig. 5a and b shows the 95% confidence regions for the parameters $\mu_{\text{max}}$, $K_S$ and $K_X$ which are bounded by the ellipsoids. The mean value for the parameter estimates lies approximately within the center (Table 3). There is correlation between $K_S$ and $\mu_{\text{max}}$ due to the long and narrow shape of the plot. However, there is less correlation between the $K_S$ and the $K_X$ (Fig. 5b), which has a more circular confidence bound compared to the parameters in Fig. 5a (Ossenbruggen et al., 1996). The correlation observed between parameter sets is often the case for Monod-based models (Holmberg, 1982). The $\mu_{\text{max}}$ and $K_S$ are better identifiable than $K_X$. Although the upper confidence limit of $K_S$ is well defined, the lower limit is shown to be unidentifiable from being numerically zero. The $K_S$ value needs to be very low in order for the model to describe low MTBE concentrations at the start and tail sections of the substrate profiles during the pulse experiments (Fig. 2c and e).

Response surface plots inclusive of the $K_S$ showed that this parameter was well identifiable due to the formation of a well-defined global minimum (not shown). However, $Y$ generally lacked sensitivity within the range explored; analysis of the response surface plots with $Y$ indicated that the 95% minimum was also indistinguishable from zero. These short-term experiments, however, are not necessarily suitable for estimation of $Y$ (Vanrolleghem et al., 1995). No advantage was gained by the inclusion of the ammonium data (Fig. 2d) as additional fit criteria for estimation of the MTBE degraders’ model parameters.

### 3.3. Ammonium oxidation kinetics

Response surface plots of any combination of $\mu_{\text{max}}$, $K_S$ and $Y$ for the ammonium oxidizers were generally flat, indicating that these parameters lacked sensitivity and thus could not be estimated. A total mass balance for the nitrogen species considering the reactions implemented in the model (Tables I and II, supplementary data) indicated that ammonium was missing during the pulsing. The Filtralite® (Gisvold et al., 2000) clay packing material of the reactor was found to sorb a significant quantity of the ammonium.
The percentage of ammonium sorbed, \( \text{NH}_4 \text{sorb} \), in the reactor during the dynamic experiments produced a minimum value of 30% from the \( w^2 \) response surface estimated by Design-Expert. The 95% confidence interval calculated using Eq. (2) with \( F_{0.05,4,26} \) was, however, unbounded. Absolute changes in \( \text{NH}_4 \text{sorb} \) did not produce relatively large changes in chi-squared.

3.3.1. Statistical tests verifying model adequacy and appropriateness
All statistical tests performed to determine the appropriateness and significance of the response surface models were found to be acceptable based on the requirements of the Design-Expert software (Figs. II and III, supplementary data). The normal plot of residuals showed a straight line through the data points, without patterns, indicating that the model residuals are normally distributed (Myers and Montgomery, 2002). The studentised residuals (\( e_i \)) showed a random scatter of the data points, indicating that model residuals are not influenced by the magnitude of the variables within the design space. Furthermore, the linear regression of \( \dot{y}^\text{pred} \) vs. \( \dot{y}^\text{exp} \) were shown to follow a straight line, with \( r^2 = 0.93 \). The p-values calculated for \( \mu_{\text{max}}, K_S, K_X \), and \( Y \) were all less than 0.1, indicating significance of the model terms (Myers and Montgomery, 2002).

3.4. Steady-state simulations

Fig. 6a–c shows the steady-state simulations of the model done with the estimated parameters (Table 3) and the experimental measurements at steady state. The estimated parameters were used to simulate the startup behavior of the system. The steady-state behavior of the system provides a greater understanding of the reactions that occur.

3.4.1. MTBE and oxygen

Fig. 6a and b shows the simulated MTBE and oxygen concentration vs. depth profiles and the experimental measurements at about 500 days of reactor operation time. The figure shows a sudden drop in the influent concentration of MTBE at the inlet of the reactor (0 m). This is mainly due to dispersion, which creates a dilution effect at the reactor’s inlet. Furthermore, the model shows that there is an increased MTBE removal capacity over time evident from a greater fraction of the inlet MTBE being removed within a smaller depth of reactor. The increased removal rate is related to increasing biomass concentrations near the reactor’s inlet (Fig. 7).

The experimental data shown (Fig. 6a and b) represents the steady state of the system at about 500 days of operation using the conditions shown in Table 1. By comparing the experimental data with the model simulations in Fig. 6a, it shows that more MTBE is removed than is possible with oxygen as the only electron acceptor. It appears that other removal processes for MTBE are occurring simultaneously in the experimental system. This issue is discussed in more details in the section below dealing with anaerobic removal of MTBE’s intermediates.

There is also some non-uniformity shown in the MTBE and oxygen experimental data as highlighted in Fig. 6b. For example, there is very little removal of oxygen and MTBE at a depth of 0.2–0.4 m; it is shown that the oxygen is also been fully consumed within this depth.

In a new experimental campaign, the PBR was decommissioned and the filtralite material washed and repacked in the system. The first set of measurements done on the reactor
showed that MTBE was removed down to less than 10 μg/L from an initial concentration of 2 mg/L within a depth of 0.2 m. The oxygen concentration in the reactor remained constant after 0.2 m (results not shown).

The mass balances using the experimental data for the sampling ports at 0.4–0.6 m showed that 1.3 g COD/m³ more oxygen was consumed than required based on stoichiometry, for the quantity of MTBE removed. The consumption of oxygen in excess of what would be required for degradation of MTBE is most likely caused by a greater endogenous respiration between ports 0.4–0.6 m than at the inlet of the reactor. Within the depth of 0.4–0.6 m the MTBE removal is only 2.3 g COD/m³ compared to 12 g COD/m³ within 0–0.2 m. After a depth of 0.6 m the MTBE experimental measurements becomes constant, and all oxygen is shown to be depleted (Fig. 6a and b).

3.4.2. Ammonium and nitrate

Fig. 6c shows the simulated steady-state ammonium and nitrate concentrations vs. depth profiles and the experimental data obtained at about 500 days. The simulations show that the ammonium oxidation capacity of the reactor increases much faster over time compared to that of MTBE. After 10 days, full oxidation of ammonium to nitrate occurs within a depth of 0.1 m. The rapid development of the nitrification capacity in the model is also consistent with the experimental findings. In a separate operational phase, than described here, ammonium was totally removed from the reactor's feed for a period of 5 months, upon re-introduction, the ammonium conversion rate to nitrate became constant, and all oxygen is shown to be depleted (Fig. 6a and b).
returned to the same as previously observed, within less than 1 month. The higher $\mu_{\text{max}}$ and lower $K_s$ are two of the most important factors governing the much faster proliferation of the nitrifiers compared to the MTBE degraders in the reactor.

There is ongoing denitrification in deeper sections of the reactor, evident from a reduction in the maximum quantity of nitrate formed (Fig. 6c). Possibly, there is also a small uptake of nitrates for heterotrophic growth. The model predicts that there is a slightly less nitrate ($0.2 \text{ g N/m}^3$), which exits the reactor than expected from the experimental data (Fig. 6c) at 500 days. The model cannot fully account for all denitrification because it appears that organic matter available is limiting. The denitrification inside the experimental system is probably also related to decaying particulate organic matter, which is physically transported with the water flow into the deeper sections of the reactor. Transport of particulate organic matter inside the reactor is not considered in the model.

3.4.3. Anaerobic removal of MTBE’s intermediates

Mass balances done at depths of 0–0.2 in the PBR showed that 4.5 g/m$^3$ of extra oxygen would have been required for a totally aerobic process. A mass balance in chemical oxygen demand (COD) equivalents applied between any two sampling ports of the PBR can be written as follows:

$$\Delta S_{\text{MTBE}} + \Delta S_{\text{NH}_4} = \Delta S_{\text{O}_2} + Y_{\text{MTBE}} \Delta S_{\text{MTBE}}$$

where $\Delta S$ represents the measured substrate change between two sampling depths. The yield of the ammonium oxidizers is disregard since it is relatively small in terms of COD.

This analysis indicates that more MTBE has been degraded than what would have been possible using oxygen as the only electron acceptor. The analytical method used showed that no TBA was detected in any of the samples. No removal of the parent MTBE molecule can be concluded under methanogenic conditions. There was also no removal of sulfates or nitrates which could have accounted for the loss of the COD equivalents observed. Therefore, other intermediates of MTBE degradation may have either been degraded in anaerobic sections within the biofilm by the heterotrophs, or may have gone undetected by the analytical method. In addition to TBA, other known intermediates of MTBE degradation are formaldehyde, 2-methyl-2-hydroxy-1-propanol, 2-hydroxy isobutyrate, 2-propanol and acetone (Fayolle et al., 2001; Wilson, 2003). Analysis for methane was done, however, none was detected. If all the missing COD equivalents in sampling ports (0–0.2 m) were converted to methane, the concentration would only be in the order of 1 µg CH$_4$/mL (water sample). However, this would not have been detected with the GC-FID instrument used. It has an absolute detection limit of around 10 µg methane.

3.5. Comparison of ATP measurements with simulated biomass profiles

Fig. 7 shows the total active biomass profiles and measured ATP profiles of the attached biofilm in µg ATP/m$^2$ (particle), at about 500 days in the operation period described here. Generally, the figure shows that the simulated biomass concentration becomes more stratified over the entire depth of the reactor with time. The active biomass concentrations are highest at the reactor’s inlet, because there the substrates are most abundant; they then decrease with increasing depth. This phenomena, were most of the activity dominates at the PBR’s inlet, has also been seen by others (Rittmann, 1982). Biomass in the deeper sections of the reactor will no longer receive sufficient substrate to maintain their formerly high concentrations; therefore, they will begin to decay over time.

Qualitatively, the ATP profile shows a similar trend as the simulated profiles; the concentration is highest at the reactor’s inlet and decreases with increasing depth. The ATP profiles are indicative of thinner biofilms, or lower microbial activity with increasing depths. It was found that the ATP concentration on a VSS basis done on the pore space biomass inside the reactor was approximately two orders or magnitude lower than for the attached biomass. This was general for the four points sampled along the reactor. The much lower ATP activity observed for measurements of the pore space biomass indicates that it is mainly composed of accumulated dead cells and inert material.

3.6. Implications of explicit exclusion of diffusion limitations in the PBR’s model

A mathematical description of the important physical and biological processes occurring in a PBR has been developed using standard reaction kinetic expressions and stoichiometry. This work represents one of the first attempts at establishing a model for a PBR in the saturated soil column compartment of AQUASIM 2.1 (Reichert, 1994). The modeling conducted is considered as a simple approach, in that, the system can be described without explicitly including external and internal diffusion aspects of a biofilm. Since most biofilm models developed are rarely ever used by engineering practitioners for reactor design or optimization due to complexity (Morgenroth et al., 2000) the approach used here may be more useful practically due to its relative simplicity. It has been shown that biofilm systems may be modeled as suspended systems; however, in these cases the half-saturation constants are actually confounded with the effects of the diffusion resistances in bioflocs. The following relationship shows how diffusion and floc diameter influences the apparent $K_s$ in a suspended system (Pérez et al., 2005):

$$K_s = 1.83 \times 10^{-2} \frac{q_{\text{max}} XD^2}{D}$$

where $q_{\text{max}}$ is the maximum removal rate in kg/(kg·s); $D$, substrate diffusion coefficient in flocs m$^2$/s; $d$, floc diameter (m) and $X$, the biomass concentration (g/m$^3$). It was reported that in activated sludge systems which are normally modeled without inclusion of biofilm diffusion resistances (Henze et al., 1987), the half-saturation constants used can be up to an order of magnitude greater than they would have been for single-cell systems (Pérez et al., 2005). As is the situation with activated sludge models and the one used in this study, the mass transfer limitation effects of diffusion will be confounded in the estimated $K_s$ values.
3.7. Model predictions and parameter estimates

The dynamic modeling approach used here has advantages for the calibration of the reactor in-situ batch determinations may add unwanted bias and uncertainties to the estimated kinetic parameters (Grady et al., 1996). Furthermore, the least-squared approach and the response surface methodology applied here adds to the current knowledge about the kinetics of MTBE degradation and uncertainty of the relevant parameters.

The \( K_S \) and \( \mu_{\text{max}} \) values for MTBE degraders have ranged widely, from approximately 0.9 to 136 gCOD/m\(^3\) and from \(<0.07\) to 0.6 d\(^{-1}\), in a temperature range 18–30 °C (Fortin et al., 2001; Waul et al., 2008a; Wilson, 2003). If mass transfer limitations due to diffusion resistances was actually significant in the reactor, the estimated \( K_S \) value would be expected to exceed the range of those reported in the literature, up to a factor of 10 (Pérez et al., 2005). However, this was not the case, the upper linear uncertainty limit for \( K_S \) was approximately 8–10 gCOD/m\(^3\) (Fig. 5a and b), and most values in the literature estimated using suspended batch cultures were less than 20 gCOD/m\(^3\). This indicates that, for the whole PBR system, diffusion limitations do not significantly affect the removal processes for MTBE. In addition to model structure, other factors such as culture history and parameter correlation may influence the values reported for both \( K_S \) and \( \mu_{\text{max}} \) (Grady et al., 1996), interpretation of \( K_S \) values from different studies can therefore be difficult. The estimated value of the \( K_S \) is also considered reasonable; the final simulated biomass concentrations of the model are limited within 10 kgCOD/m\(^3\), which is consistent for similar biofilm systems (Henze et al., 1987; Rittmann and McCarty, 2001).

The peak of the measured MTBE experimental data was shown to be consistently lower than the simulated values (Fig. 2c and e). One possible reason for this may have been an ongoing anaerobic removal of the intermediates of MTBE in the reactor, as was observed under steady-state operation. This, however, did not seem to have resulted in an increase in the estimated \( \mu_{\text{max}} \) values. Model simulations showed that no increase in \( \mu_{\text{max}} \) could have reduced the deviations between the model and simulated MTBE data; the maximum possible removal of MTBE is also governed by the oxygen available during the pulsing experiments.

In the PBR, the biomass is undergoing changes due to the varying substrate concentration over time in different sections. Therefore, it is expected that the kinetic parameters may vary over time until a steady-state substrate concentration is reached. They may also exhibit a profile in their magnitude over the depth of the reactor after the steady-state substrate profile has been reached. The parameters found, therefore, are rather specific for describing the behavior of similar biofilm systems.

3.7.1. Limitations on biomass predictions in the model

The model does not consider biomass detachment, sloughing or predation within the reactor. In addition, physical transport of particulate biomass was not addressed. Prediction of the biomass loss processes are very complicated and influenced by many factors such as, biomass growth history, substrate concentrations, hydraulic conditions, idealized biofilm assumptions and even random biofilm loss in real systems. Model simplifications may influence the predicted biomass concentration profiles over the depths of the reactor (Morgenroth and Wilderer, 2000; Telgmann et al., 2004; Van Loosdrecht et al., 1995). With such drawbacks on the current knowledge to handle model biomass systems, the application of a model as used here can be seen as a reasonable compromise for calibration of the PBR.

4. Conclusions

A model for a PBR describing biomass growth processes and substrate removal of MTBE and ammonium was developed and calibrated from dynamic modeling experiments. More specific outcomes of the study are as follows:

1. The use of least-squared analysis and evaluation of the parameter response surfaces showed that the estimated parameters were found from a global minimum. The linear uncertainty regions for growth parameters approximated from the confidence ellipsoids (Fig. 5) are approximately as follows: \(0 < K_S < 10\) mgCOD/L and \(0.12 < \mu_{\text{max}} < 0.25\) d\(^{-1}\). The 95% parameter uncertainty regions were also found to be reasonable since they are generally bounded and within the range of estimates found in the literature.

2. Response surfaces were found to be statistically significant and thus suitable for estimating the global minimum as well as the 95% parameter uncertainty regions.

3. No explicit inclusion of the external or internal biofilm diffusion resistances was implemented in the model. However, it was shown that mass transfer limitations due to diffusion resistances would actually manifest in the estimated half-saturation constants. This observation, as well as uncertainties, e.g., the modeling of biofilm detachment mechanisms in biofilm systems is necessary for an appreciation of the model. The model is considered as being a compromise to the inclusion of processes, which are not fully understood and the need for a tool, which can be readily used for the simulation of similar systems.

4. The model and the steady-state experimental data showed that competition for oxygen between MTBE degraders and ammonium oxidizers limits MTBE’s degradation in favor of ammonium oxidation. This aspect has been discussed further by application of the model (Waul et al., 2008b).

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.watres.2008.02.034.

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