“Non-linearities and upscaling in porous media“

Independent study

Modelling biofilm distribution and its effect on two-phase flow in porous media

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## Contents

1 Introduction .......................... 1
  1.1 Motivation and Goals .................. 1
    1.1.1 Goals .................................. 3
    1.1.2 Thesis Outline ................. 4
  1.2 Biofilm Structure ................. 4
  1.3 Terms and Definitions .......... 7
    1.3.1 Porous Media ............. 7
    1.3.2 Fluids .................. 8
    1.3.3 Interaction Between Fluid Phases and Porous Media .... 8
  1.4 Original Model ................. 11
    1.4.1 Dual-Continuum Concept .... 11
    1.4.2 Fluid Properties ........ 13
    1.4.3 Two-Phase Flow ........ 13
    1.4.4 Mass Balance Equations .... 13
    1.4.5 Clogging ................ 17
    1.4.6 Numerical model .... 18

2 Modifications Based on Physical Processes 21
  2.1 Mutual Dissolution of Water and CO$_2$ .... 21
    2.1.1 Solubility ............. 22
    2.1.2 Fluid Properties ...... 23
  2.2 Hydrodynamic dispersivity .... 24

3 Relating Pore Scale Growth to Macroscale Hydraulic Properties 25
  3.1 Discussion of Experimental Data ........ 25
    3.1.1 Mechanical Dispersion .... 25
    3.1.2 Diffusion in the Biofilm .... 27
    3.1.3 Intrinsic Permeability .... 28
    3.1.4 Pore Size Distribution .... 29
    3.1.5 Biofilm Structure .... 31
    3.1.6 Fluid Viscosity ...... 31
    3.1.7 Fluid Density ........ 32
3.1.8 Wetting Properties .................................................. 32
3.2 Conceptual Model of Biofilm Accumulation ..................... 32
3.3 Model Modifications .................................................. 35
  3.3.1 Intrinsic Permeability ......................................... 35
  3.3.2 Relative Permeability ......................................... 36
  3.3.3 Hydrodynamic Dispersion .................................... 41
  3.3.4 Diffusion in the Biofilm ...................................... 43
  3.3.5 Fluid Viscosity ................................................ 43
  3.3.6 Fluid Density .................................................. 44

4 Implementation of Electron Acceptor ............................. 45
  4.1 Mass Balance ...................................................... 45
  4.2 Transport .......................................................... 46
  4.3 Distribution over the Phases .................................... 46
  4.4 Distribution over the Continua ................................ 47
    4.4.1 Case 1: Uniform Film ...................................... 47
    4.4.2 Case 2: Biofilm Plugs ...................................... 49
  4.5 Consumption by Biomass.......................................... 49
    4.5.1 Biomass Growth ............................................ 49
    4.5.2 Biomass Decay ............................................ 50

5 Lysis due to Carbon Dioxide ....................................... 52
  5.1 Lysis of Suspended Cells ........................................ 52
    5.1.1 Presence of Water ......................................... 53
    5.1.2 High Pressure ............................................... 53
    5.1.3 State of Aggregation of CO\textsubscript{2} ............... 54
    5.1.4 Reduced Extracellular pH .................................. 54
    5.1.5 Reduced Intracellular pH .................................. 54
    5.1.6 Type of Microorganism .................................... 55
    5.1.7 Summary .................................................... 55
  5.2 Lysis of Attached Cells .......................................... 55
  5.3 Modelling Lysis due to CO\textsubscript{2} ............................ 56

6 Effect of Model Modifications ...................................... 58
  6.1 Parameters Affecting Biofilm Development ..................... 58
    6.1.1 Reference Simulation ...................................... 58
    6.1.2 Dispersion ................................................. 64
    6.1.3 Decay Rate ................................................. 67
    6.1.4 Injection Strategies ....................................... 69
    6.1.5 Lysis due to Dissolved CO\textsubscript{2} .................... 79
    6.1.6 Co-Injection of CO\textsubscript{2} and Electron Acceptor .... 81
  6.2 Parameters Affecting Two-Phase Flow .......................... 83
6.2.1 Pore Size Distribution Index ........................................ 83
6.2.2 Entry Pressure .......................................................... 86
6.3 Summary ................................................................. 86
7 Evaluation ................................................................. 89
# List of Figures

1.1 Biofilm used as a seal preventing CO$_2$ leakage .................................. 2  
1.2 Schematisation of biofilm growth in a porous medium .......................... 6  
1.3 Contact angle .................................................................................. 10  
1.4 Fluid interface in a capillary tube ....................................................... 10  
1.5 Dual-continuum concept ................................................................... 12  
1.6 Saturation as a function of capillary pressure ..................................... 15  
1.7 Permeability reduction due to biofilm growth ..................................... 18  
1.8 Construction of a control volume with the Box scheme ...................... 19  

3.1 Dispersion in a flat plate reactor ......................................................... 26  
3.2 Pore size reduction due to biofilm growth .......................................... 34  
3.3 Effect of increasing $\lambda_p$ on capillary pressure ................................. 37  
3.4 $\lambda_p$ as a function of biofilm accumulation ........................................ 39  
3.5 Effect of increasing $p_d$ on capillary pressure ..................................... 40  
3.6 $p_d$ as a function of biofilm accumulation .......................................... 41  

4.1 Schematisation of uniform biofilm and biofilm plug growth ................ 48  
4.2 Schematisation of flow parallel to uniform biofilm .............................. 48  

5.1 Scaling biocidal effect of dissolved CO$_2$ .......................................... 57  

6.1 Simulation domain and mesh .............................................................. 59  
6.2 Substrate consumption and biofilm accumulation during pulsed injection 63  
6.3 Effect of dispersion on biofilm accumulation ..................................... 65  
6.4 Biofilm accumulation due to an increasing $\alpha$ .................................... 66  
6.5 Biofilm and substrate distribution due to an increasing $\alpha$ ................. 68  
6.6 Biofilm accumulation due to different rates of endogenous decay ......... 69  
6.7 Biofilm distribution due to simultaneous injection ............................. 70  
6.8 Biofilm distribution due to pulsed injection ........................................ 71  
6.9 Effect of pulsed versus simultaneous injection on biofilm accumulation 72  
6.10 Effect of water injection during breaks on biofilm distribution .......... 73  
6.11 Biofilm distribution due to different length breaks ............................ 74  
6.12 Biofilm accumulation due to different length breaks ......................... 75
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.13</td>
<td>Biofilm distribution due to different length pulses</td>
<td>76</td>
</tr>
<tr>
<td>6.14</td>
<td>Biofilm accumulation due to different length pulses</td>
<td>77</td>
</tr>
<tr>
<td>6.15</td>
<td>Biofilm accumulation due to different concentrations of solutes</td>
<td>78</td>
</tr>
<tr>
<td>6.16</td>
<td>Biofilm distribution due to different concentrations of solutes</td>
<td>79</td>
</tr>
<tr>
<td>6.17</td>
<td>Biofilm accumulation due to different concentrations of electron acceptor</td>
<td>80</td>
</tr>
<tr>
<td>6.18</td>
<td>Biofilm lysis during CO$_2$ injection due to different values of $c_o$</td>
<td>81</td>
</tr>
<tr>
<td>6.19</td>
<td>Biofilm distribution during CO$_2$ injection with different values of $c_o$</td>
<td>82</td>
</tr>
<tr>
<td>6.20</td>
<td>Saturation of CO$_2$ phase and mass fraction of dissolved CO$_2$</td>
<td>82</td>
</tr>
<tr>
<td>6.21</td>
<td>Co-injection of CO$_2$ and the electron acceptor</td>
<td>83</td>
</tr>
<tr>
<td>6.22</td>
<td>Biofilm accumulation due to modifications to $\lambda_p$</td>
<td>85</td>
</tr>
</tbody>
</table>
## List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Mass balance equations of the original model</td>
<td>14</td>
</tr>
<tr>
<td>6.1</td>
<td>Biological parameters</td>
<td>60</td>
</tr>
<tr>
<td>6.2</td>
<td>Biomass attachment and detachment parameters</td>
<td>60</td>
</tr>
<tr>
<td>6.3</td>
<td>Properties of the porous medium and the biofilm</td>
<td>60</td>
</tr>
<tr>
<td>6.4</td>
<td>Fluid properties</td>
<td>61</td>
</tr>
<tr>
<td>6.5</td>
<td>Parameters for capillary pressure and relative permeability</td>
<td>61</td>
</tr>
<tr>
<td>6.6</td>
<td>Parameters for hydrodynamic dispersion</td>
<td>61</td>
</tr>
<tr>
<td>6.7</td>
<td>Properties of the aquifer</td>
<td>84</td>
</tr>
</tbody>
</table>
# Notation

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Dimension</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Greek Letters:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Gamma$</td>
<td>control volume boundary</td>
<td>[ m$^2$ ]</td>
</tr>
<tr>
<td>$\Omega$</td>
<td>control volume</td>
<td>[ m$^3$ ]</td>
</tr>
<tr>
<td>$\alpha_L$</td>
<td>longitudinal dispersivity coefficient</td>
<td>[ m ]</td>
</tr>
<tr>
<td>$\alpha_T$</td>
<td>transverse dispersivity coefficient</td>
<td>[ m ]</td>
</tr>
<tr>
<td>$\epsilon$</td>
<td>error term</td>
<td>[ - ]</td>
</tr>
<tr>
<td>$\varepsilon$</td>
<td>biofilm porosity</td>
<td>[ - ]</td>
</tr>
<tr>
<td>$\theta$</td>
<td>contact angle</td>
<td>[ ° ]</td>
</tr>
<tr>
<td>$\lambda_\kappa$</td>
<td>pore-size distribution index of Continuum $\kappa$</td>
<td>[ - ]</td>
</tr>
<tr>
<td>$\lambda_p'$</td>
<td>pore-size distribution index of biofilm-filled porous medium</td>
<td>[ - ]</td>
</tr>
<tr>
<td>$\mu_\alpha$</td>
<td>dynamic fluid viscosity of phase $\alpha$</td>
<td>[ kg/(m s) ]</td>
</tr>
<tr>
<td>$\mu_\kappa$</td>
<td>growth rate of biomass in Continuum $\kappa$</td>
<td>[ 1/s ]</td>
</tr>
<tr>
<td>$\theta_\alpha$</td>
<td>fluid density of phase $\alpha$</td>
<td>[ kg/m$^3$ ]</td>
</tr>
<tr>
<td>$\theta_b$</td>
<td>biofilm density</td>
<td>[ kg/m$^3$ ]</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>interfacial tension</td>
<td>[ N/m$^2$ ]</td>
</tr>
<tr>
<td>$\tau_{\alpha,\kappa}$</td>
<td>tortuosity of Continuum $\kappa$ for phase $\alpha$</td>
<td>[ - ]</td>
</tr>
<tr>
<td>$\phi$</td>
<td>porosity</td>
<td>[ - ]</td>
</tr>
<tr>
<td>$\phi_0$</td>
<td>porosity of porous medium unaffected by biofilm</td>
<td>[ - ]</td>
</tr>
<tr>
<td>$\phi_\kappa$</td>
<td>porosity of Continuum $\kappa$</td>
<td>[ - ]</td>
</tr>
<tr>
<td>$\phi_{p,c}, \phi'_{p,c}$</td>
<td>parameters for the calculation of $K_p$ and $K_f$</td>
<td>[ - ]</td>
</tr>
<tr>
<td><strong>Latin Letters:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C^c_\alpha$</td>
<td>concentration of component $c$ in phase $\alpha$</td>
<td>[ kg/m$^3$ ]</td>
</tr>
<tr>
<td>$D^c_{\alpha,\kappa}$</td>
<td>hydrodynamic dispersion coefficient for component $c$ in phase $\alpha$ in Continuum $\kappa$</td>
<td>[ m$^2$/s ]</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
<td>Unit</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td>------</td>
</tr>
<tr>
<td>$D_{eff,c}^w$</td>
<td>effective diffusion coefficient of component $c$ in the water phase in Continuum $F$</td>
<td>$m^2/s$</td>
</tr>
<tr>
<td>$D_{mol,c}^\alpha$</td>
<td>molecular diffusion coefficient of component $c$ in phase $\alpha$</td>
<td>$m^2/s$</td>
</tr>
<tr>
<td>$D_{mol,c}^{\alpha,\kappa}$</td>
<td>molecular diffusion coefficient of component $c$ in phase $\alpha$ in Continuum $\kappa$</td>
<td>$m^2/s$</td>
</tr>
<tr>
<td>$D_{mech}^{\alpha,\kappa}$</td>
<td>mechanic dispersion tensor of phase $\alpha$ in Continuum $\kappa$</td>
<td>$m^2/s$</td>
</tr>
<tr>
<td>$F$</td>
<td>Continuum accounting for fluid flow in biofilm</td>
<td></td>
</tr>
<tr>
<td>$P$</td>
<td>Continuum accounting for fluid flow in porous matrix</td>
<td></td>
</tr>
<tr>
<td>$K$</td>
<td>tensor of intrinsic permeability</td>
<td>$m^2$</td>
</tr>
<tr>
<td>$K$</td>
<td>isotropic intrinsic permeability</td>
<td>$m^2$</td>
</tr>
<tr>
<td>$K_0$</td>
<td>permeability of biofilm-free porous medium</td>
<td>$m^2$</td>
</tr>
<tr>
<td>$K_{min}$</td>
<td>permeability of biofilm-filled porous medium</td>
<td>$m^2$</td>
</tr>
<tr>
<td>$K_e$</td>
<td>Monod half-saturation coefficient for the electron acceptor</td>
<td>$kg/m^3$</td>
</tr>
<tr>
<td>$K_s$</td>
<td>Monod half-saturation coefficient for the substrate</td>
<td>$kg/m^3$</td>
</tr>
<tr>
<td>$M$</td>
<td>specific surface</td>
<td>$m^2/m^3$</td>
</tr>
<tr>
<td>$N$</td>
<td>ansatz function</td>
<td></td>
</tr>
<tr>
<td>$R$</td>
<td>ratio mass of the electron acceptor consumed per mass of substrate consumed</td>
<td>$kg_{e}/kg_{s}$</td>
</tr>
<tr>
<td>$S_\alpha$</td>
<td>saturation of phase $\alpha$</td>
<td>[-]</td>
</tr>
<tr>
<td>$S_{ar}$</td>
<td>residual saturation of phase $\alpha$</td>
<td>[-]</td>
</tr>
<tr>
<td>$S_e$</td>
<td>effective saturation</td>
<td>[-]</td>
</tr>
<tr>
<td>$T$</td>
<td>temperature</td>
<td>°C</td>
</tr>
<tr>
<td>$V_0$</td>
<td>pore volume of porous medium unaffected by biofilm</td>
<td>$m^3$</td>
</tr>
<tr>
<td>$V_B$</td>
<td>bulk volume</td>
<td>$m^3$</td>
</tr>
<tr>
<td>$V_b$</td>
<td>biofilm volume including biofilm pores</td>
<td>$m^3$</td>
</tr>
<tr>
<td>$V_f$</td>
<td>volume of pores within biofilm</td>
<td>$m^3$</td>
</tr>
<tr>
<td>$V_p$</td>
<td>pore volume of porous medium excluding biofilm pores</td>
<td>$m^3$</td>
</tr>
<tr>
<td>$W$</td>
<td>weighting function</td>
<td></td>
</tr>
<tr>
<td>$Y$</td>
<td>yield coefficient mass biomass produced per mass of substrate consumed</td>
<td>[-]</td>
</tr>
<tr>
<td>$X_{tox,\kappa}$</td>
<td>threshold mass fraction of CO$<em>2$ in the water phase in Continuum $\kappa$ for the calculation of $b</em>{c2,\kappa}$</td>
<td>$kg_{CO_2}/kg_w$</td>
</tr>
<tr>
<td>$X_{\alpha,c}$</td>
<td>mass fraction of component $c$ in phase $\alpha$</td>
<td>$kg_{c}/m^3_{\alpha}$</td>
</tr>
<tr>
<td>$X_{w,c}$</td>
<td>volume fraction of component $c$ in the water phase</td>
<td>$m^3_{c}/m^3_w$</td>
</tr>
<tr>
<td>$a$</td>
<td>exchange parameter</td>
<td>$s/m^2$</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>$b$</td>
<td>biomass decay rate [1/s]</td>
<td></td>
</tr>
<tr>
<td>$b_0$</td>
<td>endogenous biomass decay rate [1/s]</td>
<td></td>
</tr>
<tr>
<td>$b_{c1,\kappa}$</td>
<td>biomass decay rate due to lysis by gaseous CO$_2$ in Continuum $\kappa$ [1/s]</td>
<td></td>
</tr>
<tr>
<td>$b_{c2,\kappa}$</td>
<td>biomass decay rate due to lysis by dissolved CO$_2$ in Continuum $\kappa$ [1/s]</td>
<td></td>
</tr>
<tr>
<td>$c_w^c$</td>
<td>molar concentration of component $c$ in the wetting phase [mol/dm$^3$]</td>
<td></td>
</tr>
<tr>
<td>$c_{c1}$</td>
<td>parameter for the calculation of $b_{c1}$ [1/s]</td>
<td></td>
</tr>
<tr>
<td>$c_{c2}$</td>
<td>parameter for the calculation of $b_{c2}$ [1/s]</td>
<td></td>
</tr>
<tr>
<td>$c_{a1,2}$</td>
<td>parameters for the calculation of $k_a$ [1/s]</td>
<td></td>
</tr>
<tr>
<td>$c_{d1,2,\tilde{c}_{d,2}}$</td>
<td>parameters for the calculation of $k_d$</td>
<td></td>
</tr>
<tr>
<td>$d_e$</td>
<td>mass of electron acceptor required for endogenous decay of biomass [kg$_{ce}$/kg$_b$]</td>
<td></td>
</tr>
<tr>
<td>$d_r$</td>
<td>characteristic pore diameter [m]</td>
<td></td>
</tr>
<tr>
<td>$e$</td>
<td>exchange term [kg/(m$^3$s)]</td>
<td></td>
</tr>
<tr>
<td>$f_d$</td>
<td>fraction of cells that is biodegradable [-]</td>
<td></td>
</tr>
<tr>
<td>$g$</td>
<td>vector of gravitational acceleration $(0, 0, -g)^T$ [m/s$^2$]</td>
<td></td>
</tr>
<tr>
<td>$g$</td>
<td>(scalar) gravitational acceleration [m/s$^2$]</td>
<td></td>
</tr>
<tr>
<td>$k_{\mu}$</td>
<td>maximum substrate utilisation rate [1/s]</td>
<td></td>
</tr>
<tr>
<td>$k_a$</td>
<td>attachment function [1/s]</td>
<td></td>
</tr>
<tr>
<td>$k_d$</td>
<td>detachment function [1/s]</td>
<td></td>
</tr>
<tr>
<td>$k_d^s$</td>
<td>detachment due to shear [1/s]</td>
<td></td>
</tr>
<tr>
<td>$k_d^b$</td>
<td>detachment due to biological factors [1/s]</td>
<td></td>
</tr>
<tr>
<td>$k_{\alpha}$</td>
<td>relative permeability of phase $\alpha$ [-]</td>
<td></td>
</tr>
<tr>
<td>$k_{He}$</td>
<td>Henry coefficient [mol/(dm$^3$atm)]</td>
<td></td>
</tr>
<tr>
<td>$k_{He}^c$</td>
<td>dimensionless Henry coefficient [-]</td>
<td></td>
</tr>
<tr>
<td>$l$</td>
<td>parameter for the calculation of $\lambda_p$ [-]</td>
<td></td>
</tr>
<tr>
<td>$n$</td>
<td>unit normal vector [-]</td>
<td></td>
</tr>
<tr>
<td>$n_c$</td>
<td>parameter for the calculation of $b_c$ [-]</td>
<td></td>
</tr>
<tr>
<td>$n_k$</td>
<td>parameter for the calculation of $K_p$ [-]</td>
<td></td>
</tr>
<tr>
<td>$p_a$</td>
<td>pressure of phase $\alpha$ [N/m$^2$]</td>
<td></td>
</tr>
<tr>
<td>$p_d$</td>
<td>entry pressure [N/m$^2$]</td>
<td></td>
</tr>
<tr>
<td>$p_c$</td>
<td>capillary pressure [N/m$^2$]</td>
<td></td>
</tr>
<tr>
<td>$p_{n0}$</td>
<td>partial pressure electron acceptor in the non-wetting phase [N/m$^2$]</td>
<td></td>
</tr>
<tr>
<td>$q$</td>
<td>source/sink [kg/(m$^3$s)]</td>
<td></td>
</tr>
<tr>
<td>$r_g$</td>
<td>biomass growth [kg/(m$^3$s)]</td>
<td></td>
</tr>
<tr>
<td>$r_b$</td>
<td>biomass decay [kg/(m$^3$s)]</td>
<td></td>
</tr>
<tr>
<td>$r_a$</td>
<td>biomass attachment rate [kg/(m$^3$s)]</td>
<td></td>
</tr>
<tr>
<td>$r_d$</td>
<td>biomass detachment rate [kg/(m$^3$s)]</td>
<td></td>
</tr>
<tr>
<td>$v$</td>
<td>Darcy flux/velocity [m/s]</td>
<td></td>
</tr>
</tbody>
</table>
**x_{e,\kappa}**  
mass fraction CO\textsubscript{2} in water in Continuum \( \kappa \) scaled \(-\) by \( X_{\text{tox,}\kappa} \)

### Subscripts:
- \( \alpha \): phase, either \( w \) or \( n \)
- \( n \): non-wetting phase
- \( w \): wetting phase
- \( \kappa \): continuum, either \( p \) or \( f \)
- \( p \): Continuum \( P \)
- \( f \): Continuum \( F \)

### Superscripts:
- \( b \): biomass
- \( c \): component, either \( b, s, e \) or CO\textsubscript{2}
- \( s \): substrate
- \( e \): electron acceptor
- \( CO_2 \): CO\textsubscript{2}
- \( w \): water
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Chapter 1

Introduction

1.1 Motivation and Goals

Growing concern for global warming leads to an increased sense of urgency regarding reduction of CO$_2$ emissions. One manner to reduce these without reducing the scale of industrial activity, is to capture CO$_2$ before it enters the atmosphere. As there are currently no applications where large amounts of CO$_2$ are utilised, it is a waste product that has to be stored. For this geological formations are considered as an appropriate location.

Suitable geological formations should have a high permeability, so that CO$_2$ can be injected with minimal energy costs, combined with a high porosity, so that a large volume can be stored. To keep CO$_2$ trapped in the formation, or aquifer, a sealing caprock, a layer with low permeability, is required on top. Ideally, CO$_2$ is injected in the supercritical state, where it occupies a smaller volume than in the gas phase. This requires a high injection pressure, which results in a pressure gradient across the caprock that can cause leakage. Mitchell et al. (2008) suggest fractures can be induced during the drilling of the injection well, increasing the leakage risk in the vicinity of the well. The possibility of CO$_2$ leakage causes significant opposition to CO$_2$ storage due to perceived dangers to human life. Leakage also reduces the effectiveness of CO$_2$ storage, so that the long term benefit is uncertain.

It is suggested that a biofilm can be engineered to grow in the top of the aquifer to form a sealing layer (Cunningham et al., 2009; Mitchell et al., 2008). A biofilm consists of microorganisms that are attached to a solid surface and produce extracellular polymeric substances (EPS) surrounding them (Stewart and Franklin, 2008). Stimulating the formation of a biofilm should reduce the permeability of the aquifer, and prevent the upward flow of CO$_2$. Reductions in permeability, of up to 3 orders of magnitude, have been measured by Cunningham et al. (1991) and Taylor and Jaffé (1990), indicating the potential effectiveness of biofilm forming a sealing layer. A
schematic representation of CO₂ storage, and the use of biofilm to prevent leakage, is shown in Figure 1.1.

(a) A permeable formation, with a sealing caprock, in the vicinity of a large CO₂ source is suitable for storage.

(b) Drilling an injection well may induce fracturing in the caprock.

(c) When CO₂ is injected under high pressure, it rises in the aquifer and may leak out through cracks in the caprock.

(d) If a biofilm is grown in the top of the aquifer, this can form a sealing layer preventing CO₂ from reaching cracks near the well.

Figure 1.1: Principle for using biofilm to reduce the risk of leakage in the vicinity of the injection well.

In order to effectively apply engineered biofilms, the growth of biofilm under the conditions prevailing in the aquifer must be understood, and mathematical modelling is needed to predict and analyse the effects of biofilm growth. A model for biofilm growth in a porous medium is presented by Ebigbo (2009). The model accounts for biological factors, affecting growth and decay as well as attachment and detachment of the biofilm. Parameters have been fitted to the model based on experimental data from Mitchell et al. (2009) and Taylor and Jaffé (1990). The model can be used to model two-phase flow of CO₂ and water.

In this work some alterations are made to the model by Ebigbo (2009). Peyton (1996) suggests that more control can be exerted over the distribution of biofilm when the components required for biofilm growth, a substrate and an electron acceptor, are
injected separately. In bacterial respiration, a carbon source is converted to bacterial mass and energy, for this reaction to proceed an electron acceptor must be present. When a substrate, containing a carbon source and required nutrients, and an electron acceptor are injected separately, the location where the two components mix can be manipulated by altering the injection strategy. In the existing model, only substrate is required for biofilm growth, thus biofilm forms immediately when substrate is injected. This causes a reduction of permeability only in a limited area close to the injection well. It is desirable to be able to control biofilm distribution in order to reduce the risk of leakage over a larger area.

Biofilm formation has consequences for hydraulic properties of the aquifer besides porosity and permeability. It changes the geometry of the pores as well as reducing the pore volume. This particularly affects parameters relevant for two-phase flow. Relations expressing changes in these properties can be based on the pore scale consideration of biofilm growth.

Dissolution of CO$_2$, and its potentially harmful effects on microorganisms and biofilms, may limit the durability of a biofilm seal. Biofilm decays due to lack of nutrients, and the decay rate is increased by the presence of CO$_2$ (Kamihira et al., 1987). The role of both gaseous and aqueous CO$_2$ in the deactivation of bacterial cells is an important factor to consider.

Bio-mineralisation is suggested as an option to provide a more permanent seal by Ebigbo (2009). When calcium is injected, microorganisms in the biofilm can be stimulated to precipitate calcium carbonate, which remains in place after the biofilm itself has decayed. Modelling the distribution of biofilm is particularly important, as precipitation can only occur where biofilm is present.

1.1.1 Goals

The goals of this work can be grouped in two categories: an extension of the model to increase its practical applicability, and the refinement of the model to include several relevant physical and biological processes.

The primary goal is to enhance the applicability of the model, by the addition of a mass balance for an electron acceptor. This enables the simulation of different injection strategies, in order to manipulate biofilm growth. This should give a qualitative understanding of the control that can be exerted using different injection strategies.

Another goal is to model the effect of biofilm formation on the pore geometry. For this, a conceptual model of biofilm growth on the pore scale will be formulated, based on literature data. This should improve the representation of two-phase flow in an aquifer containing biofilm.
1.2 Biofilm Structure

Physical processes that are neglected in the original model are the mutual dissolution of CO\(_2\) and water, and the dispersion of solutes. These will be included, to investigate their effects on the model outcomes in a qualitative way. The effect of high pressure CO\(_2\) on microbial cells and biofilm, and the possibility that dissolved CO\(_2\) affects microbial cells, will be investigated using literature data. This should indicate whether the assumption that cells are deactivated only by supercritical, and not by dissolved CO\(_2\) is justified.

### 1.1.2 Thesis Outline

In the remainder of this chapter, an introduction is given to the structure of biofilms, relevant terms and definitions are discussed, and the original model by Ebigbo (2009) is presented. In Chapter 2 the implementation of physical processes, the dissolution of CO\(_2\) and water, and mechanical dispersion, is presented. Chapter 3 contains a literature study considering biofilm growth on the pore scale. A conceptual model for biofilm development is proposed, and used to formulate relations between biofilm accumulation and hydraulic parameters of the porous medium. In Chapter 4 the mass balance of the electron acceptor is presented, as well as the assumptions and constitutive relations used to solve it. Chapter 5 contains a literature study regarding the effect of dissolved and supercritical CO\(_2\), on suspended microorganisms and biofilms, and relations used to model this. The effect of modifications to the model are discussed, based on simulation outcomes, in Chapter 6. In Chapter 7 the adapted model is evaluated.

### 1.2 Biofilm Structure

In this section, the nature of biofilms is discussed, with the focus on heterogeneities of the biofilm structure. Spatially varying characteristics of the biofilm are important when modelling transport of fluids and solutes, and their interaction with the biofilm.

Stewart and Franklin (2008) describe biofilms as communities of bacterial cells that are growing attached to an interface. In the aquifer, this is the surface of the grains, but biofilms may also form on interfaces between fluids (Rockhold et al., 2005). Bacteria are microorganisms consisting of a single cell. Bacteria in biofilms produce extracellular polymeric substances (EPS) that hold the cells together. EPS compositions may vary, but the largest fraction of the EPS is made up of of polysaccharides (Mitchell et al., 2008). Microbial cells that are attached in biofilms are more resistant to stress factors imposed by their environment than suspended cells. The greater portion of cells in a natural environment will be present as part of a biofilm, which generally contains a mixture of different species (Costerton et al., 1995).
1.2 Biofilm Structure

Biofilms used to be considered as planar uniform films that had some surface roughness (Stoodley et al., 1997). This view has been adapted, as various research groups have shown biofilms to be complex 3D structures (De Beer et al., 1996; Lewandowski, 2000; Zhang and Bishop, 1994a). Descriptions of biofilm growth patterns in porous media, range from relatively uniform films to plug shaped colonies.

De Beer et al. (1994a) show there are vertical voids, they term pores, extending from the surface of the biofilm to its base. They also find a network of interconnected horizontal voids, conduits, between the biofilm and its substratum. The voids account for a large component of the total biofilm volume. Zhang and Bishop (1994a) report void volumes accounting for 99% of the total biofilm volume at the surface of the film, whereas at the base, voids account for only 30% of the biofilm volume. De Beer et al. (1996) and Stoodley et al. (1997) demonstrate that water can flow through these pores. Thus, biofilm can be considered as a porous medium, and its properties can be described using the terms elaborated in Section 1.3.

Apart from structural heterogeneity, the biological and chemical heterogeneity of biofilms are also investigated. Zhang and Bishop (1994a) show that the number of living cells relative to the total number of cells decreases, from 80% at the surface to 30% at the base of the biofilm. A decrease in the activity of the microorganisms with depth is reported by Xu et al. (1998), and Emanuelsson and Livingston (2004).

Chemical gradients exist in biofilms, these are maintained by a combination of metabolic processes and diffusive transport (Stewart and Franklin, 2008). Biofilms are described by Kim et al. (2004), and Emanuelsson and Livingston (2004), where the bacteria at the surface consume oxygen, creating a climate favorable to nitrate consuming microorganisms deeper in the biofilm. Measurements by Stewart and Franklin (2008) show zones of different microorganisms are distributed in heterogeneous colonies throughout the biofilm, as opposed to forming a layered structure.

A schematisation of biofilm growth in an aquifer is given in the top image of Figure 1.2. Biofilm takes up pore space reducing permeability, and potentially forms a sealing layer preventing fluid flow through the aquifer. In order to model the amount of biofilm present, it is important to account for the biological processes taking place in the biofilm. For this the nature of the biofilm as a porous medium must be taken into account. Fluid flow through the pores can transport substrate into the biofilm and enable more growth than when only diffusive transport takes place. Flow of CO2 in the pores of the biofilm can also make the bacteria more vulnerable to decay caused by CO2. In the bottom image of Figure 1.2 a schematisation of a biofilm on a grain surface is given. The biofilm is shown as a heterogenous structure, consisting of cell clusters with different species of bacterial cells surrounded by EPS, and significant void spaces in between.

In this work, biological heterogeneity is not investigated further. It is assumed that the biological composition of the biofilm remains relatively constant over time. The geom-
1.2 Biofilm Structure

(a) Schematisation of biofilm formation on the surface of grains. This reduces the pore space available for flow, and thus reduces the permeability of the aquifer. Biofilm can form in the shape of film, covering the grains, and as plugs that bridge pores.

(b) Schematisation of a biofilm consisting of cell clusters, containing various species of cells (small circles) surrounded by EPS (green matrix), and voids that can conduct fluid flow. The arrow between the grain surface and the dotted line indicates the thickness of the volume that is considered part of the biofilm. Particularly at the interface between the biofilm and the pore fluid, the fraction of void space relative to the volume of the cell clusters is high.

Figure 1.2: Biofilm forms on the surface of the grains reducing the permeability of a porous medium. Zooming in on the biofilm, this can also be considered as a porous medium.
etry of the biofilm, particularly the distinction between a uniform film and colonies, is significant as it affects fluid flow in the aquifer. This is considered in detail in Chapter 3. Structure and chemical gradients are important for modelling the penetration of the electron acceptor into the biofilm as considered in Chapter 4.

1.3 Terms and Definitions

In this section, terms relevant to mass transport in porous media are explained. These are classified in three groups: terms that apply to the porous media, terms that apply to the fluids, and terms describing the interaction between the two.

1.3.1 Porous Media

In this context, a porous medium is a material consisting of a solid matrix containing spaces, pores. Corey (1994) specifies that the pores must be interconnected, and large enough to contain fluid particles, but small enough so that interfacial forces (discussed in Section 1.3.3) control the location and shape of the interface between two fluids in the pore. This is the case in both geological formations and biofilms.

1.3.1.1 Porosity

Porosity, $\phi$, is the ratio of interconnected pore volume to the total volume of a porous medium.

For an aquifer this distinction is easily made. For a biofilm there exists some ambiguity on how to determine porosity experimentally. Biofilm matrix consists for over 90% of water. When image analysis is used to measure the fraction of pore space relative to the fraction of cell clusters, a cutoff value between the two must be chosen. The determination of the cutoff value will affect the resulting porosity (Lewandowski, 2000). De Beer et al. (1996) use the ability to conduct flow as a distinguishing feature between pore volume and matrix of a biofilm.

Another source of uncertainty is the determination of the total volume of the biofilm. This depends on the definition of the biofilm thickness. According to Stoodley et al. (1997) generally the height of the tallest cell clusters from the substratum can be used. This results in porosities of up to 99% in the top of the biofilm (Lewandowski, 2000; Zhang and Bishop, 1994a), as can be seen in Figure 1.2 in Section 1.2.

1.3.1.2 Intrinsic Permeability

Intrinsic permeability, $K$, describes the ease with which fluid can flow through the porous medium. This is a tensor, related to the geometry of the pores and the porosity of the medium.
1.3 Terms and Definitions

1.3.2 Fluids

The fluids that can be present in this model are water and CO$_2$.

1.3.2.1 Density

The density of a fluid, $\rho$, is defined as its mass per unit volume. This is affected by temperature and pressure as well as by the dissolution of solutes.

1.3.2.2 Viscosity

Viscosity, $\mu$, is the property by which a fluid resists deformation due to shear stress (Bear, 1979). Dynamic viscosity is the proportionality factor between shear stress and shear strain rate. This is affected by temperature, pressure and the presence of suspended or dissolved matter.

1.3.3 Interaction Between Fluid Phases and Porous Media

1.3.3.1 Saturation

The saturation, $S$, of the phase $\alpha$ is the volume fraction of the pore space that is occupied by the phase.

$$S_\alpha = \frac{V_\alpha}{\phi} \quad (1.1)$$

The effective saturation, $S_e$, is given by Equation 1.2.

$$S_e = \frac{S_w - S_{wr}}{1 - S_{wr}} \quad (1.2)$$

The residual saturation, $S_{wr}$, is described by Corey (1994) as the saturation of the wetting phase that remains present even when a very high capillary pressure is applied. Pressure cannot remove this fraction of the wetting phase from the porous medium. When the wetting phase is at a saturation below $S_{wr}$ it is considered immobile, it cannot flow through the pores.

1.3.3.2 Tortuosity

Tortuosity, $\tau$, accounts for the fact that in a porous medium flow is only possible in the pore volume, not through the grains. The distance a fluid particle travels between two points is longer than a straight line between them due to the sinuous path that is followed. The tortuosity factor is defined by Bear (1979) as the ratio between the average length of the path that streamlines follow, and the shortest distance between two parallel planes. In two-phase flow, a second phase decreases the available space, increasing tortuosity. The relation for tortuosity by Millington and Quirk (1961) is used in this model.

$$\tau_{\alpha,\kappa} = S_{\alpha,\kappa}^{7/3} \phi_{\alpha,\kappa}^{1/3}, \quad \alpha \in \{w, n\}, \quad \kappa \in \{p, f\} \quad (1.3)$$
1.3.3.3 Hydrodynamic Dispersion

Hydrodynamic dispersion, $D$, in a porous medium accounts for the spreading of a solute, driven by a concentration gradient. This is due to both effective molecular diffusion, $D_{mol,c}^{α,κ}$, and mechanical dispersion $D_{mech}^{α,κ}$. The hydrodynamic diffusion coefficient, $D_{c}^{α,κ}$, is expressed by Equation 1.4 after Bear (1979).

$$D_{c}^{α,κ} = D_{mol,c}^{α,κ} + D_{mech}^{α,κ}$$

According to Bear (1972), generally the distinction between the two processes is artificial as both occur simultaneously. At low flow velocities molecular diffusion dominates and at higher flow velocities mechanical dispersion is of greater importance.

Molecular diffusion takes place in phases composed of two or more components due to different molecular velocities of the components (Baehr, 1998). This produces a flux of particles from a higher to a lower concentration, as described by Fick’s Law. In a porous medium molecular diffusion can only take place in the pores, and is therefore related to the porosity of the porous medium, as well as the tortuosity and the saturation as discussed by Helmig (1997). In this model effective molecular diffusion is given by:

$$D_{mol,c}^{α,κ} = \hat{D}_{mol,c}^{α}τ_{α,κ}S_{α,κ}\phi_{κ}$$

Mechanical dispersion is the spreading of a pulse of solute particles due to variations in the interstitial velocity, this is the flow velocity inside the pores (Bear, 1972). This is a tensor, which can be expressed by a component parallel to the flow, longitudinal dispersivity, $α_{L}$, and a component perpendicular to this, transverse dispersivity, $α_{T}$. The mechanical dispersion coefficient is expressed in Equation 1.6 after Bear (1979).

$$D_{mech}^{α,κ} = \frac{v_{α,κ}v_{T,κ}^{T}}{|v_{α,κ}|}(α_{L,κ} - α_{T,κ}) + α_{T,κ}I|v_{α,κ}|$$

$I$ is the identity matrix, $v_{α}$ is the Darcy velocity of fluid $α$, this is the average velocity of fluid flowing through a porous medium (as given in Section 1.4).

Mechanical dispersion is scale-dependent, at different observation scales, there are other causes for variations of fluid velocity (Helmig, 1997). On the pore scale, due to viscous flow, fluid particles in the center of the pore have a greater velocity than at the walls. Flow paths through the porous medium have different lengths and the diameter of the flow path varies, causing further dispersion. On a larger scale, the heterogeneity of the medium causes additional differences in flow velocities (Welty and Gelhar, 1994).

1.3.3.4 Interfacial Tension

At the interface between two phases there is an unbalance of intermolecular forces. This results in a tensile force acting tangential to the interface, referred to as interfacial...
tension, $\sigma$. In a system with a solid phase in contact with immiscible fluids, the fluid with the lowest interfacial tension with respect to the solid is referred to as the wetting phase. $\sigma_{wn}$, refers to the interfacial tension between the fluid phases. The contact angle, $\theta$, is the angle the wetting phase makes to the solid surface as shown in Figure 1.3.

Figure 1.3: The interface between two immiscible fluids in contact with a solid surface has a contact angle $\theta$. Reprinted from Ebigbo (2009)

### 1.3.3.5 Capillary Pressure

Interfacial tension in a porous medium causes a pressure difference between the wetting and the non-wetting phase, this is the capillary pressure, $p_c = p_n - p_w$. The effect of interfacial tension on the location of the interface between two phases is illustrated in Figure 1.4.

![Figure 1.4: The location of the interface of two immiscible fluids in a capillary tube. Reprinted from Ebigbo (2009).](image)

Fluid 2 is the wetting fluid, which has a higher density than the non-wetting fluid. At point A, both fluids have the same pressure. In the capillary tube the wetting fluid is pulled upwards due to capillary pressure. There is a pressure difference between the two phases at point B.

\[
P_1^B = p^A - \varrho_1 gh \\
P_2^B = p^A - \varrho_2 gh
\]

(1.7)
The pressure difference must equal the capillary pressure for the system to be in equilibrium. The relation between capillary pressure and the radius of the capillary tube, \( r \), depends on the contact angle, \( \theta \), and the surface tension between the fluids, \( \sigma \), as given by the Young-Laplace relation:

\[
p_c = \frac{2\sigma}{r} \cos \theta \tag{1.8}
\]

### 1.3.3.6 Capillary Pressure-Saturation Relationship

A porous medium can be idealised as a bundle of capillary tubes with different pore sizes. Capillary forces determine the location of interface between the phases. The relation between saturation and capillary forces used in this work is given by Equation 1.9 after Brooks and Corey [1964].

\[
S_e = S_w - S_{wr} = \left( \frac{p_d}{p_c} \right)^{\lambda} \quad \text{for } p_c \geq p_d \tag{1.9}
\]

\( p_d \) is the entry pressure, this is the capillary pressure required for non-wetting fluid to enter the porous medium.

\( \lambda \) is the pore size distribution index. The values of \( \lambda \) theoretically can range from zero to infinity [Corey, 1994]. Natural sandstone aquifers are considered homogenous for values of \( \lambda \) greater than 1, and very homogenous for values around 4 by Bloomfield et al. (2001).

### 1.3.3.7 Relative Permeability

Relative permeability, \( k_r \), scales the intrinsic permeability to give the resistance to flow for a specific phase. When multiple fluids are present there is increased resistance to the flow of any one phase as the pore space available to this phase is reduced. The relative permeability used in this work is a function of the effective saturation \( S_e \) and pore size distribution as given by Equation 1.10.

\[
k_{rw} = S_e^{2+\lambda \frac{\lambda}{\lambda}}
\]

\[
k_{rn} = (1 - S_e)^2 \left( 1 - S_e^{2+\lambda \frac{\lambda}{\lambda}} \right) \tag{1.10}
\]

### 1.4 Original Model

In this section, the concept used in the original model is outlined, for further details please refer to the work by Ebigbo (2009).

#### 1.4.1 Dual-Continuum Concept

The model is formulated on the continuum scale; a porous medium is not considered on the level of individual solid and fluid particles, but as a continuum which can
be assigned porosity and permeability. This is obtained by averaging properties of individual molecules over a representative elementary volume (REV). The dimensions of this volume are such that it contains all phases, and its average properties do not change significantly if the size of the volume is increased.

If the entire system composed of biofilm and porous medium is modelled as one continuum, flow through the pores of the biofilm will not be considered as they are much smaller than the pores of the porous medium. Therefore, the dual-continuum concept is used. Flow is modelled separately for two continua. In Continuum \( P \), flow is through the pores of the aquifer, and biofilm is considered to be part of the solid matrix. In Continuum \( F \), flow through the pores of the biofilm is considered. This is illustrated in Figure 1.5.

![Figure 1.5](image)

Figure 1.5: Representation of the dual-continuum concept for a porous medium containing biofilm. Flow through the pores is represented by flow in Continuum \( P \), flow through the biofilm by flow in Continuum \( F \). Reprinted from Ebigbo (2009).

The volume fractions that are used in the model are listed below, after Ebigbo (2009).

- \( \phi_0 \) is the original porosity of the porous medium.
\[
\phi_0 = \frac{\text{pore volume of unaffected porous medium within REV} \ V_0}{\text{bulk volume of REV} \ V_B} \quad (1.11)
\]

- \( \phi_p \) is the porosity of Continuum \( P \).
\[
\phi_p = \frac{\text{pore volume of porous medium excluding biofilm pores} \ V_p}{\text{bulk volume of REV} \ V_B} \quad (1.12)
\]

- \( \phi_f \) is the porosity of Continuum \( F \).
\[
\phi_f = \frac{\text{volume of pores within biofilm} \ V_f}{\text{bulk volume of REV} \ V_B} \quad (1.13)
\]
\( \varepsilon \) is the biofilm porosity.

\[
\varepsilon = \frac{\text{volume of pores within biofilm } V_f}{\text{biofilm volume including biofilm pores } V_b} = \frac{\phi_f}{\phi_0 - \phi_p}
\]

### 1.4.2 Fluid Properties

CO\(_2\) is injected under high pressure so that it is present in a liquid or supercritical state of aggregation. Near the critical point the pressure, density and viscosity of CO\(_2\) change sharply. They become significantly higher than in the gas phase. Density is modelled after Span and Wagner (1996) accounting for its dependence on pressure and temperature. Viscosity is determined as function of temperature and pressure using a relation by Fenghour et al. (1998). In the water phase, density and viscosity are taken as constant values since they do not vary significantly at the temperature and pressure conditions prevailing in the aquifer. These properties are affected by the salinity of the brine and are determined after Batzle and Wang (1992). The concentration of salt in the water phase is expressed as an equivalent NaCl content, this accounts for the presence of all salts.

### 1.4.3 Two-Phase Flow

Two-phase fluid flow is given by the extended Darcy equation. The Darcy velocity, \( v_\alpha \), is the average flow velocity of a phase \( \alpha \) in the porous medium. Flow is driven by a pressure difference. Permeability and relative permeability represent the ease with which \( \alpha \) flows through the medium.

\[
v_\alpha = -\frac{k_{rr\alpha}}{\mu_\alpha} K(\nabla p_\alpha - \rho_\alpha g)
\]

Velocities in individual pores can deviate significantly from the Darcy velocity in both direction and magnitude.

### 1.4.4 Mass Balance Equations

The model has mass balance equations for water, CO\(_2\), biomass and substrate, these are given in Table 1.1.

The dispersion term \( D_\kappa \) expresses effective molecular diffusion as given in Equation 1.5 in Section 1.3. In this work a mechanical dispersivity term is added to the model. Therefore, effective molecular diffusion will be referred to as \( D_{\alpha,mol,c} \) in the remainder of this thesis.
### 1.4.4.1 Water and CO₂ Balance Equations

The equations below are used to supplement the mass balance equations for water and CO₂.

\[
\begin{align*}
1 &= S_{w,κ} + S_{n,κ} \\
\rho_{c,κ} &= \rho_{n,κ} - \rho_{w,κ} \\
k_{rα,κ} &= k_{rα,κ}(S_{w,κ}) \\
\end{align*}
\]

(1.16)

The pressures in Continuum $P$ and Continuum $F$ are assumed to be equal, so fluid exchange between the continua is instantaneous. The density and viscosity of the fluids are the same in both continua. The wettability of the biofilm is assumed to be the same as the wettability of the porous medium. The capillary pressure is equal at the interface between the continua, so the effective saturations of the two continua are related as shown by Equation 1.17

\[
\begin{align*}
p_{c} &= p_{d,p}S_{c,p}^{\frac{1}{\lambda_p}} \\
S_{c,f} &= \left(\frac{p_{d,f}}{p_c}\right)^{\frac{1}{\lambda_f}} \text{ if } p_c > p_{d,f} \\
S_{c,f} &= 1 \text{ otherwise.}
\end{align*}
\]

(1.17)

It is assumed that $p_{d,f} > p_{d,p}$ and that $p_{d,p}$ is constant with biofilm accumulation. Thus the non-wetting phase will invade Continuum $P$ first, and only enter Continuum $F$ if the capillary pressure exceeds the the entry pressure, $p_{d,f}$. This is when the wetting phase saturation in Continuum $P$ is low, as illustrated in Figure 1.6.

### 1.4.4.2 Biomass Mass Balance Equation

The biomass mass balance equation is formulated by assuming all biomass in Continuum $P$ is suspended in water and can be expressed by the concentration $C_{w}^{b}$ [kg/m³].

---

**Table 1.1: Mass balance equations of the original model after Ebigbo (2009)**

<table>
<thead>
<tr>
<th>Mass balance equations</th>
<th>Continuum $P$</th>
<th>Continuum $F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>$\frac{\partial(\phi_p S_{w,p} + \phi_f S_{w,f})}{\partial t} + \nabla \cdot (\rho_w [\mathbf{v}<em>{w,p} + \mathbf{v}</em>{w,f}]) = q_w$</td>
<td>$\frac{\partial(\phi_p S_{n,p} + \phi_f S_{n,f})}{\partial t} + \nabla \cdot (\rho_n [\mathbf{v}<em>{n,p} + \mathbf{v}</em>{n,f}]) = q_n$</td>
</tr>
<tr>
<td>CO₂</td>
<td>$-\nabla \cdot (D_p \nabla C_{w}^{b}) = q_{w}^{b} - e_{w}^{b}$</td>
<td>$\frac{\partial(\phi_p S_{w,p} C_{w,p}^{b})}{\partial t} + \nabla \cdot (C_{w}^{b} \mathbf{v}<em>{w,p}) = q</em>{w,p}^{b} - e_{w,p}^{b}$</td>
</tr>
<tr>
<td>Biomass</td>
<td>$\frac{\partial(\phi_p S_{w,p} C_{w,p}^{b})}{\partial t} + \nabla \cdot (C_{w,p}^{s} \mathbf{v}<em>{w,p}) = q</em>{w,p}^{s} - e_{w,p}^{s}$</td>
<td>$\frac{\partial(\phi_f S_{w,f} C_{w,f}^{b})}{\partial t} + \nabla \cdot (C_{w,f}^{s} \mathbf{v}<em>{w,f}) = q</em>{w,f}^{s} + e_{w,f}^{s}$</td>
</tr>
<tr>
<td>Substrate</td>
<td>$\frac{\partial(\phi_p S_{w,p} C_{w,p}^{s})}{\partial t} + \nabla \cdot (C_{w,p}^{s} \mathbf{v}<em>{w,p}) = q</em>{w,p}^{s} - e_{w,p}^{s}$</td>
<td>$\frac{\partial(\phi_f S_{w,f} C_{w,f}^{s})}{\partial t} + \nabla \cdot (C_{w,f}^{s} \mathbf{v}<em>{w,f}) = q</em>{w,f}^{s} - e_{w,f}^{s}$</td>
</tr>
</tbody>
</table>

---
In Continuum $F$ all biomass is attached in the form of biofilm with a constant biofilm density $\rho_b$ and porosity $\varepsilon$. Since supercritical CO$_2$ is a biocide there is no biomass suspended in the gas phase. Biomass that has decayed is no longer accounted for in the model, it disappears.

The source term for biomass is comprised of biomass growth rate, $r_{g,\kappa}$, and a decay rate, $r_{b,\kappa}$, and external sources, $\dot{q}^b$.

$$ q^b = r_{g,\kappa} - r_{b,\kappa} + \dot{q}^b $$ (1.18)

The growth rate of biomass, $r_{g,\kappa}$, is a function of the amount of biomass present. In Continuum $P$ this is the concentration of biomass in water, in Continuum $F$ this is the product of the volume fraction of biofilm, $\phi_f/\varepsilon$, and the density of the biofilm.

$$ r_{g,p} = \mu_p \phi_p S_{w,p} C^b_w $$
$$ r_{g,f} = \mu_f (\phi_f/\varepsilon) \rho_b $$ (1.19)

The growth rate coefficient, $\mu_\kappa$, depends only the concentration of substrate, and is modelled using single Monod kinetics as shown in Equation (1.20)

$$ \mu_\kappa = k_\mu Y \frac{C^s_{w,\kappa}}{K_s + C^s_{w,\kappa}} $$ (1.20)

$k_\mu$ is the maximum substrate utilisation rate, $Y$ [kg biomass/kg substrate] is the yield coefficient, which accounts for the amount of substrate used to produce new biomass; the remainder is used for cell maintenance. $K_s$ is the Monod half saturation coefficient.
this is the value that $C_{n,\kappa}^*$ has when $\mu_\kappa = k_p Y / 2$. The values of these parameters are the same for cells in suspension and cells in the biofilm.

Biomass decay is the reduction of the amount of biomass due to various causes. This is in part due to consumption of intracellular material by respiration, but also to other factors like predation by protozoa as discussed by van Loosdrecht and Henze (1999). The endogenous decay rate coefficient, $b_0$, is a lumped parameter and constant in both continua.

Lysis is the death of microbial cells due to harsh environmental conditions. Supercritical CO$_2$ can have a damaging effect on microbial cells both in suspension and in the biofilm. The coefficient expressing decay due to lysis, $b_{c1,\kappa}$, is dependent on the saturation of the CO$_2$ phase as shown in Equation 1.21. This is a lumped parameter accounting for a variety of mechanisms by which CO$_2$ affects microbial cells.

$$b_{c1,\kappa} = c_{c1} S_{n,\kappa}^{nc}$$  \hspace{1cm} (1.21)

$c_{c1}$ and $n_c$ are empirical values. The overall decay rate coefficient can be expressed as the sum of $b_0$ and $b_{c1,\kappa}$ as given in Equation 1.22.

$$b_\kappa = b_0 + b_{c1,\kappa}$$  \hspace{1cm} (1.22)

The total decay rate is given in Equation 1.23.

$$r_{b,p} = b_p \phi_p S_{w,p} C_w^b$$  \hspace{1cm} (1.23)

$$r_{b,f} = b_f (\phi_f / \varepsilon) \theta_b$$

Biomass exchange between the two continua involves the attachment of suspended cells to the biofilm and detachment of cells or aggregates from the biofilm.

$$e^b = e_f^b = -e_p^b = k_{a1} \phi_p S_{w,p} C_w^b - k_{d} (\phi_f / \varepsilon) \theta_b$$  \hspace{1cm} (1.24)

Attachment is modelled by Equation 1.25.

$$k_a = c_{a1} + c_{a2} \phi_f / \varepsilon$$  \hspace{1cm} (1.25)

$c_{a1}$ and $c_{a2}$ are empirical parameters. As more biofilm accumulates it plays a more important role in attachment by increasing the adsorption rate of suspended cells and increasing the probability of straining.

The rate of detachment is affected by shear stress acting on the biofilm and by the growth rate of the biofilm as shown in Equation 1.20. Detachment due to shear forces is represented by the term $k_d^\mu$, where the water pressure gradient $|\nabla p_w|$ accounts for the shear forces of the water on the biofilm. Biofilms with a higher growth rate are considered more susceptible to shear, which is expressed in the term $k_d^\mu$. 
\[ k_d = \frac{c_{d,1}(\phi_p S_{w,p}|\nabla p_w|)^{n_d} + c_{d,2} \mu_f (\phi_f / \varepsilon) \theta_b}{k_d^*} \] (1.26)

\[ c_{d,1}, c_{d,2}, \text{ and } n_d \] are empirical parameters. It is assumed the proportionality between \( k_d^* \) and growth rate varies with the volume fraction of biofilm, \( \phi_f / \varepsilon \):

\[ c_{d,2} = \tilde{c}_{d,2} \phi_f / \varepsilon \] (1.27)

This is due to the assumption that in a when a high volume fraction of biofilm is present, the substrate is distributed more unevenly in the biofilm, giving a more heterogenous biofilm and more detachment.

### 1.4.4.3 Substrate Mass Balance Equation

The consumption of substrate by biomass is given by the sink term, \( q_{w,s}^a \).

\[ q_{w,s}^a = -r_{g,s} / Y \] (1.28)

The growth rate of biofilm, \( r_{g,s} \) is given in Equation [1.19] and \( Y \) is the yield coefficient for mass of biomass produced per mass of substrate consumed.

Substrate exchange between the two continua is primarily due to diffusion, driven by a concentration gradient, as fluid exchange between the continua is considered to be instantaneous. Equation [1.29] is used to model substrate exchange between the two continua.

\[ D_{w}^{eff,s} = D_{w}^{eff,s} \frac{A_{pf}}{L}(C_{w,p} - C_{w,f}) \] (1.29)

\( D_{w}^{eff,s} \) is the effective diffusion coefficient of the substrate in the biofilm. \( L \) is a characteristic length over which the concentration difference exists, for which the characteristic pore radius \( d_r / 2 \) is used. \( A_{pf} \) is the specific interfacial area between the two continua, which is approximated by the specific surface, \( M \), of the porous medium.

### 1.4.5 Clogging

The permeability of Continuum \( F \) and Continuum \( P \) are added to give the total permeability of the two continua. This is done by summing the mass fluxes through each continuum.

\[ \mathbf{J} \cdot \mathbf{A} = \mathbf{J}_p \cdot \mathbf{A}_p + \mathbf{J}_f \cdot \mathbf{A}_f \] (1.30)

\( \mathbf{J} \) is the total mass flux of a fluid and \( \mathbf{A} \) is the total cross-sectional area through which the flux travels. Pressure gradients and fluid properties, as well as the cross sectional areas are equal for the continua, therefore, the total flow can be described using the Darcy equation as shown in Equation [1.31].

\[ -K \frac{\theta_w}{\mu_w} \nabla p_w \cdot \mathbf{A} = -(K_p + K_f) \left( \frac{\theta_w}{\mu_w} \nabla p_w - \theta_w \mathbf{g} \cdot \mathbf{A} \right) \] (1.31)
When little biomass is present the total permeability is primarily determined by the permeability of Continuum $P$, as this is significantly greater than the permeability of the biofilm. Permeability changes in Continuum $P$ are related to the changes in porosity of Continuum $P$ due to biofilm growth. The reduction of permeability of Continuum $P$ is given by Equation 1.32.

$$
\frac{K_p}{K_0} = \left( \frac{\phi_p - \phi_{p,c}}{\phi_0 - \phi_{p,c}} \right)^{n_k} \quad \text{if } \phi_p > \phi_{p,c}
$$

$$
K_p = 0 \quad \text{otherwise}.
$$

(1.32)

$n_k$ is an empirical parameter that depends on the geometry of the porous medium. $\phi_{p,c}$ is the critical porosity where the permeability of Continuum $P$ is zero and flow is only through Continuum $F$. Continuum $F$ is assigned a permeability $K_{min}$ based on experimental data. The permeability of Continuum $F$ increases linearly with the volume of biomass. It is zero when no biofilm is present, and rises to $K_{min}$ when the porosity of Continuum $P$ is $\phi_{pc}$. Figure 1.7 illustrates the development of permeability as a function of the pore space occupied by the biofilm.

![Figure 1.7: Permeability decrease due to formation of biofilm fitted to data from Cunningham et al. (1991). Permeability of Continuum $P$ decreases sharply until $\phi_p = \phi_{pc}$ when the permeability of Continuum $P$ is zero. The permeability of Continuum $F$ increases from zero when $\phi_p = \phi_0$ to $K_{min}$ when $\phi_p = \phi'_{pc}$. Reprinted from Ebigbo (2009).](image-url)

1.4.6 Numerical model

The mass balance equations form a coupled system of non-linear differential equations that is implemented in the multiphase flow simulator MUFTE-UG (see
This section describes the temporal and discretisation, and the linearisation that are used.

### 1.4.6.1 Temporal discretisation

For temporal discretisation a fully implicit Euler scheme is used. A differential equation in time is written as a finite difference as shown in Equation 1.33

\[
\frac{u^{t+\Delta t} - u^t}{\Delta t} = f(u^{t+\Delta t})
\]  

(1.33)

\(\Delta t\) is the time-step size. The value of the variable \(u\) at the new time step \(t + \Delta t\) is evaluated using the value of the function \(f\) at the new time step. Thus, the nodal value of \(u_n^{t+1}\) depends not only on values in the previous time step, but also on the values of neighboring nodes at the new time step. The system of equations is solved for all nodes simultaneously. This makes the method more stable, but also computationally more expensive.

### 1.4.6.2 Spatial discretisation

Spatial discretisation is done using the box method. The domain is divided into elements of a finite size by a finite element mesh. Each element is divided into a number of subcontrol volumes equal to the number of nodes of the element. This is done by constructing a finite volume mesh that connects the barycenter of each element with the midpoint of the lines bounding the element as shown in Figure 1.8. The result of this is that there is a control volume, box, around each node.

![Box Method Diagram](image)

**Figure 1.8:** Construction of a control volume with the box scheme. Reprinted from Ebigbo (2009)

The box method is a vertex centered finite volume scheme, the values of the variables are solved exactly at each node and interpolated between the nodes using linear ansatz functions. The method is isoparametric, the geometry of the domain is approximated with the same ansatz functions as the values of the variables. The ansatz function \(N_j\)
has a value 1 at node $j$ and zero at all the other nodes. The approximated function of
a variable $u$, $\tilde{u}$ is given as:

$$\tilde{u} = \sum_{j=1}^{n} u_j N_j$$  \hspace{1cm} (1.34)

Gradients are described by ansatz functions in the same manner.

$$\nabla \tilde{u} = \sum_{j \in \eta_i} N_j (u_j - u_i)$$  \hspace{1cm} (1.35)

The weak form of the balance equations is obtained by integrating over a control
volume $\Omega$. The weak form implies that the equation holds for the volume over which
it is integrated, but not necessarily at each point in the integration volume. When the
approximated function is inserted in the weak form this results in an error term $\epsilon$. By
applying appropriate weighting functions the total error over the domain $\Omega$ becomes
zero.

$$\int_{\Omega} W_j \epsilon d\Omega = 0$$  \hspace{1cm} (1.36)

The weighting function $W_j$ has a value of 1 inside the control volume of the node $j$,
and a value of zero outside it. The box method is locally mass conservative, thus for
every control volume the mass balance equation holds. All mass transfer is accounted
for over the boundaries of the finite volume box, and is thus conserved between the
nodes.

1.4.6.3 Linearisation

The system of differential equations is non-linear, due to various relations like the
relation for relative permeability as a function of saturation and intrinsic permeability
as a function of porosity, that are non-linear. The system of balance equations can be
expressed as a non-linear function $f$ of a set of variables $u$.

$$f(u) = 0$$  \hspace{1cm} (1.37)

This system is linearised using the Newton-Raphson method as follows:

$$u_{r+1} = u_r - \left( \frac{\partial f}{\partial u} \right)^{-1} \cdot f(u_r)$$  \hspace{1cm} (1.38)

$r$ is the current iteration step and $\frac{\partial f}{\partial u}$ is the Jacobian matrix. The Jacobian matrix
is evaluated numerically using the software package UG, which contains various linear
solvers.
Chapter 2

Modifications Based on Physical Processes

Modifications are made to represent some physical processes that are neglected in the original model. These are the dissolution of CO$_2$ in water, and vice versa, and the use of mechanical dispersivity in the hydrodynamic dispersion tensor.

2.1 Mutual Dissolution of Water and CO$_2$

Supercritical CO$_2$ has a low solubility in water, and its dissolution was initially neglected. Dissolution of CO$_2$ increases the density of the water phase, which plays a role in the long term storage of CO$_2$. According to Javadpour (2009), this only becomes important over longer timescales, for which this model is not intended. On shorter timescales, flow is driven primarily by injection pressure and the density difference between the fluids. A more important reason to include the dissolution of CO$_2$ is the possibility that it affects the growth and decay rates of the biofilm, this is considered in Chapter 5.

CO$_2$ in aqueous solution gets hydrated to form carbonic acid, H$_2$CO$_3$. The equilibrium for this reaction favours molecular CO$_2$, and most of the CO$_2$ in the water phase is not hydrated (Soli and Byrne, 2002). Dissociation of H$_2$CO$_3$ causes a reduction in the pH of the water phase. This change is affected by the composition of the brine and by the mineralogy of the aquifer, however, pH changes are neglected in this thesis.

The solubility of water in supercritical CO$_2$ is very low (Lu et al., 2009). Dissolution of water in the CO$_2$ phase may become important when the saturation of the water phase is very low, and the residual water dissolves in the supercritical CO$_2$. This process can lead to complete formation dry out in the vicinity of the injection well according to Pruess and Muller (2009). The dissolution of water contained in biofilms that are exposed to supercritical CO$_2$ has been observed by Mun et al. (2009). This
leads to increased interaction between the supercritical CO\(_2\) and the biofilm, and an increased lysis of the biofilm.

The exchange of mass between the fluid phases implies that the phase composition is no longer constant, this adds additional terms to the mass balances used in the original model. The new mass balance equations for CO\(_2\) and water are given in Equations 2.1 and 2.2 respectively. In order to solve these equations, further relations are used that are discussed in this section.

\[
\frac{\partial}{\partial t} \left( \phi_p S_{n,p} X_{n,p}^{CO_2} + \phi_f S_{n,f} X_{n,f}^{CO_2} \right) \varrho_n + \left[ \phi_p S_{w,p} X_{w,p}^{CO_2} + \phi_f S_{w,f} X_{w,f}^{CO_2} \right] \varrho_w \\
+ \nabla \cdot \left( \varrho_n [X_{n,p}^{CO_2} v_{n,p} + X_{n,f}^{CO_2} v_{n,f}] \right) + \nabla \cdot \left( \varrho_w [X_{w,p}^{CO_2} v_{w,p} + X_{w,f}^{CO_2} v_{w,f}] \right) \\
- \nabla \cdot \varrho_n (D_{n,p}^{CO_2} \nabla X_{n,p}^{CO_2} + D_{n,f}^{CO_2} \nabla X_{n,f}^{CO_2}) \\
- \nabla \cdot \varrho_w (D_{w,p}^{CO_2} \nabla X_{w,p}^{CO_2} + D_{w,f}^{CO_2} \nabla X_{w,f}^{CO_2}) \\
= q_n
\]

\[
\frac{\partial}{\partial t} \left( \phi_p S_{w,p} X_{w,p}^w + \phi_f S_{w,f} X_{w,f}^w \right) \varrho_n + \left[ \phi_p S_{n,p} X_{n,p}^w + \phi_f S_{n,f} X_{n,f}^w \right] \varrho_n \\
+ \nabla \cdot \left( \varrho_n [X_{n,p}^w v_{n,p} + X_{n,f}^w v_{n,f}] \right) + \nabla \cdot \left( \varrho_w [X_{w,p}^w v_{w,p} + X_{w,f}^w v_{w,f}] \right) \\
- \nabla \cdot \varrho_n (D_{n,p}^w \nabla X_{n,p}^w + D_{n,f}^w \nabla X_{n,f}^w) \\
- \nabla \cdot \varrho_w (D_{w,p}^w \nabla X_{w,p}^w + D_{w,f}^w \nabla X_{w,f}^w) \\
= q_w
\]

\(X_{\alpha,\kappa}^c\) is the mass fraction of component \(c\) in phase \(\alpha\) in Continuum \(\kappa\). When the mass balance of CO\(_2\) or water is considered for one continuum, an exchange term \(e_{\alpha}^c\) is included in the right hand side of the equation, to account for the exchange of the component between Continuum \(F\) and Continuum \(P\). Thermodynamic equilibrium is assumed between the water and the gas phase, exchange is instantaneous and requires no exchange term.

### 2.1.1 Solubility

The solubility of CO\(_2\) is implemented using a model proposed by [Duan and Sun](2003). They compare the results to experimental data, including the range of temperature, pressure, and salinity conditions that can be expected to prevail in a formation for CO\(_2\) storage, to verify the predictions from the model.
2.1 Mutual Dissolution of Water and CO$_2$

The exchange of dissolved CO$_2$ between the two continua, $e^w_{CO_2}$, is assumed to be driven by a concentration gradient. The exchange is similar to the exchange of substrate in the original model (refer to Section 1.4) and is given by Equation (2.3).

$$e^w_{CO_2} = -e^w_{CO_2} = e^w_{CO_2} = D_{eff,CO_2} M \frac{d_r}{2} \left( X^w_{CO_2} - X^f_{CO_2} \right)$$  (2.3)

$D_{eff,CO_2}$ is the effective diffusion coefficient of CO$_2$ in the biofilm, $d_r$ and $M$ are the characteristic pore diameter and the specific surface of the porous medium respectively.

The solubility of water in supercritical CO$_2$ is implemented as a constant value. Different methods to determine this value are considered. Duan et al. (2008) suggest the partial pressure of water in the supercritical CO$_2$ can be approximated by the saturated vapour pressure. Spycher et al. (2003) suggest formulae based on data regressions over experimental datasets. Lu et al. (2009) compare these approaches and find the solubility values predicted by Spycher et al. (2003) are several times higher than those predicted by Duan et al. (2008). Both formulas are used to determine the composition of the CO$_2$ phase, and combined with different equations of state, to evaluate the densities of the resulting mixtures. The disparities in the resulting densities are relatively small, due to the low solubility of water. At the pressure range expected in the model, the solubility of water is relatively constant, and the value used is an average of the two predicted by the relations cited above. The concentration of water in the CO$_2$ phase is the same in both continua, thus the exchange term $e^w_g$ equals zero.

2.1.2 Fluid Properties

In the original model, the viscosity of the water phase is taken as a constant value, which is adjusted for the salinity. Dissolution of CO$_2$ in the water phase affects the viscosity of the water phase, however, this effect is negligible compared to the effect of dissolved salts according to Enick (1992). The viscosity of the water phase without suspended biomass is modelled as in the original model. Adjustments that are made to account for suspended biomass are discussed in Chapter 3.

Dissolution of CO$_2$ in water results in a denser fluid. The density of water phase containing dissolved CO$_2$ and salts is modelled after Garcia (2001).

The solubility of water in the CO$_2$ phase is constant and has a very low value. The density of the CO$_2$ phase neglecting dissolution of water, determined using the equation of state by Span and Wagner (1996), falls within the range of densities obtained by Lu et al. (2009) where dissolved water is accounted for. Thus it appears acceptable to determine the density of the CO$_2$ phase neglecting its water content. The same is done for the viscosity of the CO$_2$ phase.
2.2 Hydrodynamic dispersivity

In the original model, only molecular diffusion is included in the term for hydrodynamic dispersivity. The model is adapted to also include the mechanical dispersion term $D_k^{\text{mech}}$ (as given in Equation 1.4 in Section 1.3). The relative importance of diffusion and mechanical dispersion depends on flow velocity; especially in vicinity of the injection well, mechanical dispersion may become significant. An increase in the hydrodynamic dispersivity tensor results in increased spreading of a pulse of injected solutes. As this model will be extended to model pulsed injection, the effect of mechanical dispersion on biofilm accumulation may be significant.

Another reason to include mechanical dispersion is to account for the changes in hydrodynamic dispersivity in Continuum $P$ due to biofilm formation. A reduction in porosity of Continuum $P$ results in a decrease in effective molecular diffusion as given by Equation 1.5 in Section 1.3. Experimental data, discussed in Section 3.1, indicate that biofilm accumulation increases the total hydrodynamic dispersivity of a porous medium by increasing the mechanical dispersivity. A relation between biofilm accumulation and mechanical dispersivity is proposed in Section 3.3.

Mechanical dispersivity is implemented for both phases in Continuum $P$. In Continuum $F$ the flow velocities are lower making dispersion less important relative to diffusion, therefore, dispersion is neglected in Continuum $F$. This assumption is also made by Rockhold et al. (2005) for a comparable model considering flow in biofilms. Effective diffusion inside the biofilm, and the effect of biofilm formation on mechanical dispersivity coefficients, are considered in Chapter 3.
Chapter 3

Relating Pore Scale Growth to Macroscale Hydraulic Properties

Biofilm accumulation modifies the pore geometry of a porous medium, which is reflected in macroscale hydraulic properties such as porosity, permeability, diffusivity, pore size distribution, and entry pressure. In the following section, a discussion of literature regarding biofilm growth on the pore scale is presented. Based on this hypotheses regarding biofilm growth patterns are combined, to formulate a conceptual model of biofilm accumulation. Using this model, as well as existing biofilm models, relations between biofilm volume fraction and hydraulic parameters are formulated in Section 3.3.

3.1 Discussion of Experimental Data

Research in the field of biofilm growth in porous media is primarily stimulated by applications in enhanced oil recovery and water treatment. Experiments show a wide range of apparently conflicting outcomes regarding the structure of biofilms and the location in the pores where biofilm preferentially grows. The outcomes of experiments are to a great extent influenced by experimental conditions, such as substrate loading, which is evaluated by Rittmann (1993). In the following discussion, various laboratory experiments reported in literature are examined in order to gain an understanding of the changes induced in a porous medium by biofilm growth.

3.1.1 Mechanical Dispersion

Dispersion is observed to increase due to biofilm growth by Sharp et al. (1999), Seymour et al. (2007) and Seifert and Engesgaard (2007) among others. Dispersion is a macroscale phenomenon caused by differences in the interstitial velocity, as described in Section 1.3. The effects of biofilm accumulation on dispersion have been studied using visual observation of flow cells, tracer breakthrough curves, and magnetic
3.1 Discussion of Experimental Data

Resonance microscopy (MRM). MRM can be used to measure interstitial flow velocities. Sharp et al. (1999) suggest biofilm formation can increase dispersivity by increasing the tortuosity of the porous medium, and by the development of dead end pores and preferential flow paths. This is observed in transparent flow cells when a tracer dye is injected, as shown in Figure 3.1. In the flow cell containing biofilm, most of the injected dye flows through a few open channels. A large portion of the flow cell conducts no flow, and no dye enters these zones. The flow velocities in the channels of the cell containing biofilm, are higher than the flow velocities in the pores of the clean flow cell. The time it takes the dye front to reach the position shown in Figure 3.1 is significantly longer for the clean flow cell. In these experiments the total flow rate is constant. When biofilm creates areas where no flow can take place, the velocity in the remaining open channels increases.

(a) Flow of an injected tracer dye in a clean transparent flat plate cell. Time since injection tracer: 160 seconds.

(b) Flow of an injected tracer dye in a transparent flow cell with biofilm accumulation. Most dye flows through preferential flow paths, large areas contain no dye indicating little to no fluid flow. Time since injection tracer: 45 seconds.

Figure 3.1: Images of a tracer dye flowing through a flat plate reactor. Reprinted with permission from Schultz (2010)

Using MRM images, Metzger et al. (2006) study the formation of preferential flow paths. They suggest the flow pattern shifts from a random sphere bed packing flow to Poiseuille flow. Seymour et al. (2007) use MRM to analyse the probability that a fluid particle travels a certain distance in a specific time in a system where biofilm grows. Biofilm accumulation results in a higher probability of smaller displacements, and the maximum displacement value increases. The former is due to fluid getting trapped in dead end pores, the latter is due to increased flow velocities in the unclogged channels. Based on this they argue that biofilm growth increases the complexity of the porous medium to such an extent that the regular dispersion equation (Equation 1.6 in Section 1.3) is not suitable to describe it.
Tracer breakthrough curves, from tests like the one shown in Figure 3.1, are modelled by various researchers using Equation 1.6 for mechanical dispersion. Results for the change in longitudinal dispersivity, $\alpha_L$, vary, which can be attributed to differences in experimental conditions. Taylor and Jaffe (1990) observe increases in $\alpha_L$ of 3 orders of magnitude for a column experiment with a high accumulation of biofilm. Under low substrate loading, Bielefeldt et al. (2002) find an initial increase in $\alpha_L$, followed by a decrease as more biofilm forms, to a value slightly above the starting value. At higher substrate loading, they find a uniform increase of 1 order of magnitude. Seifert and Engesgaard (2007) report a linear increase that only started when a significant volume of biomass is present in the system. The observed increase is 1 order of magnitude, however, they suggest it may reach up to 40 times the initial value for more severely clogged areas. The majority of the observations indicate that $\alpha_L$ starts to increase as soon as biofilm forms, and continues to rise until the medium reaches a quasi-steady state permeability (Bielefeldt et al., 2002; Metzger et al., 2006; Sharp et al., 2005).

The experiments cited above consider only longitudinal dispersivity. Yarwood et al. (2006) report an increase in transverse dispersivity, $\alpha_T$, and a decrease in $\alpha_L$. In their experiments, water can flow around the zone containing biofilm through clean porous medium. This reduces the amount of flow, and the flow velocities, in the biofilm affected zone, giving a reduction in $\alpha_L$. These findings may not be representative for dispersion inside the biofilm affected porous medium.

Sorption to the biofilm is shown to play an important role in slowing the transport of solutes like tetrachloroethene by Nambi et al. (2003). Stewart (1998) suggests that sorption to biofilms mainly affects the transport of ionic solutes. Breakthrough curves of oxygen and methane from in situ tests, measured by Semprini and McCarty (1991) indicate these substances are not retarded with respect to the flow of the bulk fluid, implying sorption can be neglected for those solutes.

Tracer tests generally show that injected tracer remains in the medium for a longer time when the medium contains biofilm. In some cases, not all tracer is recovered, as observed in experiments by Taylor and Jaffe (1990). This is attributed to diffusion of the tracer inside the biofilm cell clusters. Seifert and Engesgaard (2007) suggest that increases in hydrodynamic dispersion are caused by both an increase in $\alpha_L$ and by diffusion of the tracer into the biofilm cell clusters.

### 3.1.2 Diffusion in the Biofilm

Diffusion in biofilms occurs both inside the pores (De Beer et al., 1996), and in the cell clusters (Emanuelsson and Livingston, 2004; Stewart, 2003). Experiments from Zhang and Bishop (1994b) to determine effective diffusion coefficients in biofilms,
show that the value of the diffusion coefficient varies significantly with the depth of the biofilm. As discussed in Section 1.2, biofilms have a homogenous structure, with large variations in properties that affect diffusion, such as composition and porosity.

A review of experimental data for diffusion of different types of solute in biofilms is presented by Stewart (1998). Based on this, ratios for the effective diffusion coefficient in the biofilm, $D_{\text{eff,c}}$, relative to the molecular diffusion coefficient in water, $D_c$, are recommended for different types of solute. These values are constants that are representative for the entire thickness of biofilm, and can be used for reaction diffusion interactions (Stewart, 1998).

### 3.1.3 Intrinsic Permeability

The effect of biomass accumulation on intrinsic permeability depends on the mechanism of permeability reduction, as well as on the total amount of biomass that is present. Many authors study the reduction in permeability as a function of time throughout the experiment, and the total amount of biomass is determined only at the end. For modelling fluid flow in this model, it is important to relate the mass of biofilm present to the permeability changes. In this section, experiments describing the changes in permeability and biofilm accumulation over time are discussed.

Different mechanisms of clogging are reported. When a biofilm forms on a surface, microbial cells produce EPS, as discussed in Section 1.2. The volume of EPS is shown to be much greater than the volume of bacterial cells, for some types of microorganism by Thullner et al. (2002). For those species, EPS filling the pore space causes the reduction in permeability. On the other hand, bacteria that do not produce EPS have been shown to also cause clogging by Vandevivere and Baveye (1992). This is attributed to the formation of cell aggregates in suspension, these get strained in the pore throats and plug the pores of the medium.

Biofilm accumulation results in a minor reduction in permeability in the first phase of many experiments, followed by a period where permeability falls rapidly, until a quasi-steady state is reached (Bielefeldt et al., 2002; Cunningham et al., 1991; Seifert and Engesgaard, 2007) (refer to Figure 1.7 in Section 1.4). According to Cunningham et al. (1991), the thickness of the biofilm follows a sigmoidal growth curve, a conclusion based on observations and measurements during biofilm growth. The patterns of permeability reduction and biofilm accumulation suggest that permeability can be correlated to the volume of biofilm. Initially microbial cells are injected and attach to the grains, which causes little reduction in pore volume and permeability. As they grow and the thickness of the biofilm becomes enough to significantly affect the porosity of the porous medium, the permeability drops sharply. When a threshold volume is reached the permeability becomes relatively constant.
When the quasi-steady state is reached, the amount of biomass in the porous medium is not necessarily constant. Seifert and Engesgaard (2007) suggest the porosity continues to decrease, with little further effect on the total permeability. Observations by Cunningham et al. (1991) suggest that biofilm thickness remains constant, but the density of the biofilm can increase. Bielefeldt et al. (2002) suggest that at the steady state, growth is balanced by biofilm losses due to detachment and decay. Oscillations of the permeability at the quasi-steady state situation are reported by various authors (Dupin and McCarty, 1999; Stewart and Kim, 2004). Dupin and McCarty (1999) suggest that in a biofilm affected medium, most flow occurs through preferential pathways, as shown in Figure 3.1. When these get clogged, this causes a temporary reduction in total permeability, which lasts until the plug is removed by increased pressure gradient over the channel. These conclusions are based on experiments with a constant total flow rate, where biofilm accumulation leads to a higher pressure gradient over the reactor. This results in higher shear forces that cause erosion and removal of plugs in preferential flow paths.

The value of the steady state permeability is similar for media with different initial permeabilities (Bielefeldt et al., 2002; Cunningham et al., 1991). In media with a greater porosity, a greater accumulation of biofilm forms than in media with a smaller porosity, giving a similar final permeability. Thullner et al. (2002) show that when the substrate and the electron acceptor are injected at different locations and allowed to mix through diffusion this results in a smaller reduction of permeability near the injection point than when the substrate and the electron acceptor are co-injected.

The nature of clogging depends in part on the type of microorganism studied. For microorganisms that produce significant amounts of EPS, permeability reduction can be related to the decrease in porosity due to biofilm accumulation. Increases in the volume fraction of biofilm reduce permeability, until a state is reached where permeability remains relatively constant. The processes playing a role in maintaining the quasi-steady state are related to interactions between biofilm accumulation and mass transport and hydrodynamics (Cunningham et al., 1991).

### 3.1.4 Pore Size Distribution

The effect of biofilm on the pore size distribution of a porous medium is dependent on the size of the pores in which the biofilm initially forms and the growth pattern of the biofilm. It is suggested that the pore size distribution may affect the intrinsic permeability by Assouline and Or (2008). The relation between the pore size distribution index and relative permeability is expressed by Equation 1.10 in Section 1.3. Different observations relevant to changes in the pore size distribution are considered in this section.
Torbati et al. (1986) observe biofilm forming plug shaped colonies in the larger pores of sandstone cores, which is attributed to the greater availability of nutrients in larger pores. In these experiments, there is no flow through the sample whilst the biofilm grows, thus there are no shear forces and substrate transport is limited to diffusive fluxes. Subsequent measurements of pore size distribution index show a reduction in the number and size of peaks, indicating biofilm makes the cores more homogeneous.

Paulsen et al. (1997) perform experiments in replicas of sandstone cores, using constant flow rates similar to those near an injection well. They observe biofilm forming an initially relatively uniform film in all pores, with flagellar movements at the interface of the biofilm and the pore fluid. The formation of streamers is also described by Stoodley et al. (2005), particularly for turbulent flow. Paulsen et al. (1997) describe that as growth progresses, cell clusters develop that are larger in the areas where the flow rate is lower. The formation of a uniform biofilm in the first stage of growth followed by greater accumulation in more sheltered areas is also observed by Cunningham et al. (1991). Paulsen et al. (1997) note the formation of channels in the biofilm at locations where the flow rates are particularly high. The formation of channels and preferential flow paths is also reported by authors studying dispersion (Metzger et al., 2006; Schultz, 2010; Sharp et al., 2005).

Stoodley et al. (2005) and Sharp et al. (1999) describe aggregates being sloughed off the biofilm due to shear forces and transported by the bulk fluid. Suspended cells and aggregates are observed to travel at the same velocity as the water front in the main bulk flow, but closer to the pore walls larger cell aggregates are slowed down and trapped (Paulsen et al., 1997). Therefore, in addition to a greater chance of straining, the probability of attachment is also higher in smaller pores.

The observations described above suggest a relatively uniformly distributed film, with thin streamers at the surface, is likely to form initially. As the biofilm develops, shear forces can promote formation of larger colonies in more sheltered areas of the medium. Attachment and detachment mechanisms favor an accumulation of biofilm in the smaller pores, and the development of preferential flow paths through larger pores that remain relatively unclogged.

A demonstration project where an engineered biofilm is grown on a field scale is studied by Cunningham et al. (2003). An area where the grain size is coarser experiences a smaller reduction in permeability than the surrounding finer grained material. It is suggested the coarser grained area contributes to channeling of the flow through the system. This prevents pressure from building up over the areas with a finer grain size. In this situation, it appears that biofilm accumulation has a more significant effect in smaller pores.
3.1.5 Biofilm Structure

The structure of a biofilm is a much debated aspect of biofilm growth. The two extreme structures considered for biofilms in porous media are: a uniform thickness film over the grains, and the formation of colonies in the form of plugs bridging across the pores. For both forms, the biofilm has an internal geometry, as described in Section 1.2, containing pores through which fluid flow is possible. It is important to consider the distinction between the structures, since biofilms forming plugs require a lower volume fraction of biomass to cause a given reduction in permeability (Thullner et al., 2002; Vandevivere et al., 1995).

Stoodley et al. (1999) observe that biofilms grown in a regime with low flow velocities tend to form a relatively uniform film over the grains, whereas higher flow velocities result in a more heterogeneous structure. The effect of both types on pressure gradient appears to be the same. Bielefeldt et al. (2002) suggest that at a higher flow rate biofilm with a greater density forms, giving a higher accumulation of biomass but not a greater reduction in permeability of the porous medium.

Microscopic observations of biofilm development by Dupin and McCarty (1999) reveal simultaneous formation of a uniform film as well as colonies that span across the pores. Often, immediately after a colony plugs a pore, a biofilm forms downstream. This is attributed to a reduction in shear stress caused by the plug.

Rittmann (1993) reviews experiments indicating either uniform film or biofilm colony formation. He argues that the spatial distribution of biofilm can be predicted from the substrate loading conditions. At very low substrate loading there is not enough substrate to sustain a film over the grains and separate colonies develop. When the available substrate increases these can join to form a more uniform biofilm.

These results indicate that the supply of substrate and the shear forces play a significant role in determining the structure of the biofilm. It appears that a biofilm formed in an aquifer for CO$_2$ storage will consist of both a film, with some degree of surface roughness and filaments protruding from the surface, and larger colonies bridging pores.

3.1.6 Fluid Viscosity

The viscosity of the formation brine is affected by suspended biomass. There is little experimental data concerning the effect of microbial cells and aggregates on the viscosity of water. Rockhold et al. (2002) suggest the Einstein Equation, given in Equation 3.1, is suitable to model the change in viscosity. This equation relates the change in viscosity of a fluid to the volume fraction of suspended material.

\[
\mu = \mu_0 \left(1 + \frac{5}{2} X_{o}^{c}\right)
\]

\(X_{o}^{c}\) is the volume fraction of a component \(c\) in the fluid \(\alpha\). \(\mu_0\) is the viscosity of the fluid without suspended matter.
3.1.7 Fluid Density

The average density of microbial cells is very close to that of water, 1.1 kg/m$^3$ \cite{Rockholdetal2002}. Thus the effect of dissolved biomass will be negligible.

3.1.8 Wetting Properties

Biofilm growth is reported to cause changes in the wettability of the porous medium. This is suggested to be due to a change in the contact angle or the surface tension by \cite{Rockholdetal2002} and \cite{Yarwoodetal2006}. Experimentally, neither group measured a significant effect of suspended biomass on the surface tension of water. \cite{Rockholdetal2002} suggest that some species of microorganisms, not tested by them, may produce surfactants in their metabolism.

3.2 Conceptual Model of Biofilm Accumulation

Based on the experimental findings discussed in Section 3.1, a conceptual model is presented here that describes the development of a biofilm in an aquifer. The location where biofilm forms and its structure are key factors to assess biofilm induced changes of some hydraulic parameters. These factors are explicitly addressed in this section, considering the three stages of the sigmoidal growth curve of biomass in a porous medium, as described by \cite{Cunninghametal1991}. The microorganisms that are injected are assumed to be a species that produce EPS, which accounts for a significant fraction of the volume of the biofilm.

Injected microbial cells can cause clogging of the smallest pores by straining \cite{Vandevivereetal1995}. The first attachment of microbial cells may occur on all grains. In this stage, biofilm forms a relatively uniform thin film with some surface heterogeneities and filaments in all pore sizes \cite{Cunninghametal1991, Paulsenetal1997, Stoodleyetal2005}. The thickness of the biofilm may be enough to clog some smaller pores. The minimum pore size present increases, as the smallest pores become clogged. This is illustrated in image (a) of Figure 3.2.

In the next stage of growth, the film thickens and colonies may form, protruding predominantly into pores that are sheltered from shear forces \cite{Cunninghametal1991, Nambietal2003, Paulsenetal1997} as shown by images (b) and (c) of Figure 3.2. When colonies plug a pore, the reduction in shear forces may induce rapid biofilm growth downstream \cite{DupinMcCarty2000}. In pores with high flow velocities, channels may form in the biofilm \cite{Paulsenetal1997}. Preferential flow paths, conduits which conduct most of the flow, are likely to develop \cite{DupinMcCarty2000, Seymouretal2004, Shapetal1999}. Sloughing can erode lumps of material from the biofilm. Colonies clogging pores...
may be washed away entirely as pressure builds up [Dupin and McCarty, 2000; Stewart and Kim, 2004; Stoodley et al., 2005], this effect is mainly observed for colonies in the preferential flow paths [Dupin and McCarty, 2000]. Detached biofilm aggregates may re-attach to biofilm or get caught in smaller pores by straining [Paulsen et al., 1997; Vandevivere et al., 1995].

When a state is reached where the permeability remains relatively constant, the pore size distribution will also be relatively constant, as the thickness of the biofilm does not increase significantly [Bielefeldt et al., 2002; Cunningham et al., 1991]. Detachment and attachment cause changes in the locations of flow paths as paths get plugged and unplugged, but this does not affect the pore size distribution significantly. This state is represented by images (c) and (d) of Figure 3.2.

The effect of biofilm accumulation on the pores of a porous medium is summarised schematically in Figure 3.2. The overall range of pore sizes decreases, fast relative to the mass accumulation of biofilm at first, more slowly as a larger number of pores gets clogged. Less biofilm is required to clog smaller pores, thus formation of a relatively uniform biofilm will cause the smallest pores to be clogged with only little biofilm accumulation. This results in an increase in the minimum pore radius. As accumulation proceeds, the pore size of all pores is reduced by the formation of biofilm. Observations suggest larger colonies are formed preferentially in pores experiencing low shear stresses, these tend to be smaller pores with lower flow velocities. Biofilm can be transported from larger pores to smaller pores by detachment and attachment mechanisms. Sloughing appears more significant in larger pores and creates preferential flow paths, this limits the reduction of the maximum pore size. Straining and attachment have a greater probability to take place in smaller pores, causing these to remain clogged to a greater extent than the larger pores.

During CO\textsubscript{2} injection, the CO\textsubscript{2} phase enters largest pores first due to capillary forces. The biofilm in these pores will be lysed to a greater extent, as supercritical CO\textsubscript{2} is a biocide [Zhang et al., 1998]. This tends to enhance the situation where the smaller pores are plugged with biomass to a greater extent than the larger pores.

Biofilm grows when an electron acceptor and a substrate are both present. If pulsed injection strategies are used, the biofilm grows in the zone where the electron acceptor and the substrate mix through dispersion, as studied by Nambi et al. (2003) and Thullner et al. (2002). By adjusting the timing of the pulses, the distance from the well and the spatial distribution of the biofilm can be manipulated [Peyton, 1996; Semprini and McCarty, 1991].
3.2 Conceptual Model of Biofilm Accumulation

(a) Injected bacterial cells attach to the pore walls and form a thin, relatively uniform thickness, film in all pores. Thin filaments may form at the surface of the biofilm. Some of the smallest pores may get clogged by straining of bacterial cells.

(b) The biofilm begins to grow and accumulate volume. The thickness of the film increases relatively uniformly, and thicker colonies may form in sheltered pores. The smallest pores get clogged reducing the range of pore sizes.

(c) The volume of biofilm increases faster, biofilm thickens, and more colonies form increasing the heterogeneity of the biofilm. Smaller pores get clogged and all pore sizes are reduced to some extent.

(d) The volume of biofilm is relatively constant. As pressure builds up over clogged pores, biofilm detaches and preferential flow paths form mainly in larger pores. Suspended aggregates strain in small pores keeping these clogged.

Figure 3.2: Schematisation of the pores of a porous medium in which biofilm grows. The pores are represented by circles of different sizes. Biofilm grows attached to the walls of the pores. The mass of biofilm follows a sigmoidal growth curve, cells are injected and attach (a), initially accumulation is relatively slow (b), the growth rate speeds up (c) and reaches a quasi-steady state level (d). The overall effect is a reduction in the range of sizes of the unclogged pores.
3.3 Model Modifications

In this section, several modifications to the original model are presented, based on the conceptual model outlined in Section 3.2. Various authors propose different models for biofilm growth and its effects on porous media. Alternative methods of relating intrinsic and relative permeability to biofilm accumulation are considered. The effect of biofilm on hydrodynamic dispersion is also considered important to add to the model, as well as the effect of suspended biomass on fluid viscosity.

3.3.1 Intrinsic Permeability

Many models represent biofilm growth by assuming a uniform film formation in all the pore sizes (Cunningham et al., 1991; Taylor and Jaffé, 1990). These models give good results for coarser sands but have been criticised to be inadequate for fine grained material (Bielefeldt et al., 2002; Thullner et al., 2002; Vandevivere et al., 1995). They can underpredict the change in permeability because they do not account for formation of plugs bridging pores or clogging of pore throats by straining (Thullner et al., 2002; Vandevivere et al., 1995).

Thullner et al. (2002) simulate biofilm growth in pore network models assuming either a uniform film or plugs. These models show that the same reduction in permeability requires less biomass for plug formation. In pore network models, pores are idealised as tubes with a given length and constant diameter. A plug only needs to fill a fraction of the length of the pore, whereas a film needs to fill the entire pore (Dupin et al., 2001). The difference in the amount of biofilm required for clogging by a film and by colonies therefore also depends on the length of the pores in the model. This affects the relations that are suggested between permeability reduction and biomass accumulation.

Clement et al. (1996) present a parametric model that makes no assumptions regarding the structure of biofilm. They derive a relationship that links porosity to permeability based on the effect of biomass accumulation on the pore radius. This is criticised as assuming film formation (Seifert and Engesgaard, 2007). The assumption is made that biofilm forms preferentially in the larger pores. In the conceptual model used in this work, it is assumed biofilm forms in all pores, and that smaller pores are affected to a greater extent.

Vandevivere et al. (1995) propose a model, which combines the effects of formation of uniform biofilm and clogging. Clogging in this context is the deposition of discrete multicellular aggregates at and blockage of pore constrictions. For this model, the volume of biomass at which the biofilm is most unstable is an input parameter. This is considered to be the volume of biomass at which many pores switch from porosity reduction by film formation to being clogged. This parameter depends on both the
3.3 Model Modifications

The matrix of the porous medium and the detachment rate of the biofilm. Detachment is described as one of the least understood processes (Cunningham et al., 1991) and known to play an important role in the development of biofilms (Clement et al., 1996; Vandevivere et al., 1995). Factors affecting detachment include shear forces (Rockhold et al., 2002), nutrient availability (Rockhold et al., 2005), the structure of the biofilm (Picioreanu, 1999) and the thickness of the biofilm. Nutrient availability and shear forces can vary due to pulsed injection of substrate and an electron acceptor in this model. Injection of CO₂ alters shear forces, and supercritical CO₂ has a biocidal effect (Kamihira et al., 1987). This suggests that the volume of biomass at which the biofilm is most unstable may not be a constant value for the situations that this model must represent.

Thullner and Baveye (2008) demonstrate the importance of considering flow through the biofilm pores, allowing for substrate transport in the biofilm. This allows the biofilm to accumulate to a thickness that corresponds to experimental observations. Flow through the pores of the biofilm is also important to account for the steady state permeability observed by Bielefeldt et al. (2002), Cunningham et al. (1991) and Taylor and Jaffé (1990).

Based on the discussion above, the method used to model permeability reduction in the original model (described in Section 1.4) appears appropriate. It accounts for rapid reductions in total permeability due to biofilm accumulation in Continuum $P$. A steady state constant total permeability is possible due to flow in Continuum $F$. The model has been fitted to experimental data from Cunningham et al. (1991), Taylor and Jaffé (1990) and Mitchell et al. (2009). A similar approach is suggested by Rockhold et al. (2002) in the form of a composite media model, where total permeability is also the sum of permeability of the matrix and permeability of the biofilm. No specific growth pattern is assumed in the permeability relation. The biofilm structure mainly affects parameters important for two-phase flow, thus it appears more suitable to express these in the formulation for relative permeability.

3.3.2 Relative Permeability

Changes in relative permeability due to biofilm accumulation are studied for flow in soils under unsaturated conditions. Models developed for this generally assume biofilm to initially clog the smaller pores, as these contain water and nutrients. Wissmeier and Barry (2009) underline the importance of accounting for changes in pore geometry as well as changes in pore volume when modelling unsaturated flow.

Mostafa and Van Geel (2007) present a model where the biofilm is considered as one of the three phases filling the pore space. The effective saturation is the sum of the effective biofilm saturation and the effective water saturation. This approach is verified by Soleimani et al. (2009) using the data from Taylor et al. (1990). This
3.3 Model Modifications

conflicts with the conceptual model used in this work. In Continuum $P$ biofilm is considered part of the solid matrix, not part of the pore space.

To account for observed changes in the capillary pressure-saturation curve, Rockhold et al. (2005) suggest modelling a reduction in surface tension as a result of suspended biomass. Maggi and Porporato (2007) complement this approach by also scaling pore size distribution index and entry pressure as a function of biofilm thickness. In aquifers considered for CO$_2$ storage, the presence of minerals and possibly hydrocarbons affects interfacial forces. The surface tension of pure water is very unstable, and is reduced by the presence of most organic contaminants (Corey, 1994). The relation between interfacial forces and the volume of biomass is therefore not easily defined, and the additional uncertainty may outweigh the benefit of this assumption. It is preferred to relate pore size distribution index and entry pressure to the volume of biofilm, as these modifications can be based on changes in pore geometry suggested by the conceptual model.

3.3.2.1 Pore Size Distribution Index

Biofilm development, as outlined in the conceptual model in Section 3.2, is expected to clog smaller pores to a greater extent than larger pores. Wissmeier and Barry (2009), demonstrate that clogging of the smaller pores results in an increase in pore size distribution index, $\lambda_p$. The effect of an increase in $\lambda_p$, using Equation 1.9 (refer to Section 1.3), on the capillary pressure is shown in Figure 3.3

![Figure 3.3: Increasing the value of $\lambda_p$ results in a shift of the $p_c$-$S_e$ curve. For any value of effective saturation the capillary pressure of the Continuum $P$ is reduced.](image)

It is suggested by Maggi and Porporato (2007) to correlate $\lambda_p$ to fractal dimension, $D$, in order to formulate a relation between biofilm volume fraction and $\lambda_p$. Mater-
3.3 Model Modifications

Materials with a fractal geometry contain regular patterns that can be observed at different scales. Natural porous media, such as soils, can be described by fractal geometry (Tyler and Wheatcraft, 1990; Xu and Sun, 2002). The fractal dimension of a soil can be related to $\lambda_p$, but the relation used depends on the geometry considered. Tyler and Wheatcraft (1990) regard pores as capillary tubes with uniform diameter along their entire length, which results in the relation $\lambda = 2 - D$. Xu and Sun (2002) consider pores as spheres, which yields $\lambda = 3 - D$. The latter is the correlation used by Maggi and Porporato (2007). They suggest Equation 3.2 to model the effect of biofilm growth on $D$.

$$D(\phi_0 - \phi_p) = D_0 + (D' - D_0) \left( \frac{\phi_0 - \phi_p}{\phi_0 - \phi_{c}} \right)$$ (3.2)

$D_0$ is the fractal dimension of the clean soil, and $D'$ is the fractal dimension when the biofilm has reached a quasi-steady state. It is noted by Maggi and Porporato (2007) that the form of the relationship is arbitrary and can be improved if more empirical data is available.

The fractal description is most often applied to soils (Hunt, 2001; Hunt and Gee, 2002), and the capillary tube approach is shown to be valid for values of $\lambda$ in the range of 0.05-0.29 (Tyler and Wheatcraft, 1990). Theoretically, $\lambda$ can take values from zero to infinity (Corey, 1994). In typical sandstone aquifer cores values of $\lambda$ have been determined in the range from 0.002 to 2.27, but they are suggested to reach up to 4 for uniformly graded sandstones (Bloomfield et al., 2001). Correlating $\lambda_p$ to $D$ limits the range of possible values for $\lambda_p$. Particularly the relation $\lambda = 2 - D$ is less suitable to express values that may need to be accounted for by the model.

Alternative descriptions for the effect of biofilm formation on $\lambda$ are scarce in literature. The relationship for $\lambda_p$ proposed in this work is based on the conceptual model of biofilm growth. The increase of $\lambda_p$ is greater when low volumes of biomass are present. In this stage the smaller pores are plugged, and only a small volume of biofilm is required to plug these pores. When these are clogged, more biofilm is required to clog successively larger pore sizes. When a steady state permeability is reached, $\phi_p = \phi_{p,c}$, $\lambda_p$ attains a constant value. Reports of the same quasi-steady state permeability for media with different initial permeabilities (Bielefeldt et al., 2002; Cunningham et al., 1991), suggest the quasi-steady state hydraulic parameters play a more significant role than the initial hydraulic parameters. Therefore, the quasi-steady state value of $\lambda_p$ is similar for soils with different initial values of $\lambda_p$. Equation 3.3 gives the relation between the volume of biofilm, $(\phi_0 - \phi_p)$, and $\lambda_p$, implemented in this model.

$$\lambda_p = \lambda_{p,0} + (\lambda'_p - \lambda_{p,0}) \left( \frac{\phi_0 - \phi_p}{\phi_0 - \phi_{p,c}} \right)^l$$ (3.3)

$l$ and $\lambda'_p$ are empirical parameters that can be fitted to data. $l$ should have a value less than 1, giving in a greater change in $\lambda_p$ when the biofilm volume fraction is low. $\lambda'_p$
is value $\lambda_p$ takes when a quasi-steady state is reached. The variation of $\lambda_p$ with the volume fraction of biofilm present, for $\lambda'_p = 3.0$ and $l = 1/3$, is shown in Figure 3.4.

Figure 3.4: $\lambda_p$ is plotted as a function of the fraction of pore volume occupied by biofilm using Equation 3.3. The gradient of the curve decreases as the volume fraction of biofilm increases until the critical porosity of Continuum $P$, $\phi_{p,c} = 0.6 \phi_0$ is reached. The parameters used are: $\lambda'_p = 3.0$, $l = \frac{1}{3}$, $\phi_0 = 0.22$.

### 3.3.2.2 Entry Pressure

Entry pressure is the value of capillary pressure where the non-wetting phase can enter the largest pores of the porous medium, as discussed in Section 1.3. Entry pressure, $p_d$, is related to the maximum pore radius, $r_a$, as given by Equation 3.4.

$$ p_d = \frac{2\sigma}{r_a} \cos \theta $$

(3.4)

Biofilm accumulation is likely to cause a reduction of the pore diameter for all pores, and thus causes an increase in the entry pressure. Using Equation 1.9 (refer to Section 1.3), an increase in entry pressure leads to an increase in the capillary pressure of the porous medium, if the effective saturation is constant, as shown in Figure 3.5. Maggi and Porporato (2007) propose Equation 3.5, which is similar in form to Equation 3.2, to model the reduction of entry pressure with biofilm accumulation.

$$ p_d(\phi_p - \phi_0) = p_{d,0} + (p'_d - p_{d,0}) \left( \frac{\phi_0 - \phi_p}{\phi_0 - \phi_c} \right) $$

(3.5)

$p_{d,0}$ is the entry pressure of the clean soil, and $p'_d$ is the entry pressure when the biofilm has reached a quasi-steady state. This relationship appears to contradict their
assumption of biofilm forming preferentially in the smallest pores. When biofilm grows preferentially in smaller pores, the entry pressure is mainly affected when the volume fraction of biofilm is high, instead of rising linearly with \((\phi_0 - \phi_p)\).

Wissmeier and Barry (2009) show calcite precipitation, which occurs mainly in the smallest pores, results in only a minor increase in entry pressure. Assouline and Or (2008) propose a relationship to estimate the change in maximum pore radius, \(r_a\), due to compaction in sandstones given by Equation \(3.6\):

\[
\frac{r_a}{r_{a0}} = \left(\frac{1 - \phi_p}{1 - \phi_0}\right)^{-3.82}
\]  

(3.6)

\(r_a\) is related to the air entry pressure \(p_d\) through the capillary law as expressed in Equation \(3.4\). \(r_{a0}\) is the maximum pore radius for the clean porous medium. Assuming that the interfacial tension, \(\sigma\), and the contact angle, \(\theta\) are constant, the relationship can be written as:

\[
p_d = p_{d0} \left(\frac{1 - \phi_p}{1 - \phi_0}\right)^{3.82}
\]  

(3.7)

This relation gives a slightly slower increase as the porosity is reduced initially, as shown in Figure \ref{fig:3.5}. This corresponds to the change expected based on the growth pattern described in the conceptual model. The maximum value that \(p_d\) attains is less than the entry pressure of Continuum \(F\) so that the assumption \(p_{d,f} > p_{d,p}\) is not violated.

Assouline and Or (2008) found this equation reproduced the relative change in pore size with decreasing porosity quite well, using data for sedimentary rocks from
3.3 Model Modifications

Figure 3.6: $p_d$ is plotted as a function of the fraction of pore volume occupied by biofilm using Equation 3.6. Using the equation, the value of $p_d$ increases until the entire pore space is filled with biofilm, however, when the critical porosity of Continuum $P$ is reached, the permeability of Continuum $P$ is zero and changes in $p_{d,p}$ no longer affect fluid flow. $p_{d0} = 1.0 \times 10^4$ Pa, $\phi_0 = 0.22$, $\phi_{p,c} = 0.6 \phi_0$.

Arns et al. (2005).

For Continuum $F$, the entry pressure is estimated assuming the wetting properties of the biofilm are the same as those of the porous medium. Due to biofilm heterogeneity (as described in Section 1.2) a wide range of pore sizes can be expected. Reported pore diameters are in the range of $50\mu$m, De Beer et al. (1994b) to $2.7 \mu$m Zhang and Bishop (1994a). Due to the large variation in these numbers biofilm entry pressure is approximated by a constant value. The pore radii of the biofilm appear to be in a range of 1 to 2 orders of magnitude smaller than the pore radii of the porous medium. The entry pressure of Continuum $F$ is taken as 1 order of magnitude larger than the entry pressure for Continuum $P$.

3.3.3 Hydrodynamic Dispersion

The observations discussed in Section 3.1 indicate biofilm formation increases mechanical dispersion by creating stagnant areas in the porous medium where flow velocities are very low, and preferential flow paths with high velocities. Solutes can also diffuse into the biofilm, causing further dispersion of an injected pulse of solute. The dual-continuum model accounts for both changes in the pore geometry of Continuum $P$ and exchange of solute between the bulk fluid and the biofilm. Changes in pore geometry affect the mechanical dispersion term of Continuum $P$, diffusive transport of solutes inside the biofilm is expressed in effective diffusion term for Continuum $F$. 


3.3.3.1 Mechanical Dispersion

On the pore scale, mechanical dispersivity is caused by differences in flow velocity inside the pores. It is suggested by Rittmann (1993) that biofilm accumulation makes the surface of the grains more irregular. This can give a larger spread of flow velocities inside the pores. Biofilm also causes the formation of preferential flow paths and stagnant zones (Sharp et al., 1999), this increases dispersion due to fluid particles traveling along flow paths with different flow velocities. Therefore, the value of the dispersion tensor should depend on the volume of biofilm present. Several factors have to be considered when using laboratory observations to formulate a relation for field scale dispersion. These are: the scale dependence of dispersivity, the longitudinal and transverse components of dispersion, and the relation between dispersivity and porous medium properties.

A limitation in using laboratory data to determine mechanical dispersivity, is the scale dependence of the dispersivity parameter $\alpha$. This has been found to be several orders of magnitude greater in field than in laboratory experiments for the same porous medium. Therefore, Gelhar et al. (1992) deem laboratory experiments unsuitable to predict field scale dispersivity. On the field scale, dispersivity is caused by larger scale variations in permeability, and the value of $\alpha$ increases with the range of interest. The effect of biofilm formation on dispersivity, on the field scale, is not a topic that has received much attention in literature.

Mechanical dispersion includes dispersivity both parallel and perpendicular to the flow direction. Experimental conditions by Bielefeldt et al. (2002), Sharp et al. (1999), Seifert and Engesgaard (2007) and Taylor and Jaffe (1990) are representative only for dispersivity in the direction parallel to flow. The effect on transverse dispersivity is not studied in these experiments.

The influence of porous medium properties such as particle size and pore-size distribution on dispersivity coefficients has not fully been studied using experimental data according to Costanza-Robinson and Brusseau (2006). There are few literature references regarding models that distinguish between diffusion and dispersion and less that account for changes in dispersivity of a porous medium. Taylor and Jaffe (1990) argue that changes in dispersivity are important to include in order to model mass transport in the biofilm affected medium. They derive an expression for dispersivity based on the geometry of a porous medium. Using the assumption that a uniform thickness biofilm forms, increases in dispersivity are predicted of up to 3 orders of magnitude for a thick biofilm accumulation. This is an underprediction of the dispersivities observed by them, but it is significantly higher than the values reported by most authors, which are in the range of a factor 2 to 8 increase according to Seifert and Engesgaard (2007).
In a study of cementation by mineral precipitation, Cochepin et al. (2008) assume that the dependence of $\alpha$ on porosity is relatively small. The effect of reduced porosity on the effective molecular diffusion coefficient, $D_{mol,s}^{\alpha}$, is considered, but changes in mechanical dispersivity are neglected. Rockhold et al. (2005) consider a separate mechanical dispersion and a molecular diffusion term when studying changes in hydraulic properties of a porous medium induced by biofilm growth. In this model, $\alpha$ is considered constant, and effective molecular diffusion is a function of saturation and porosity.

In order to incorporate dispersivity changes, the dispersivity coefficient $\alpha$ is modelled to increase linearly with the volume fraction of biofilm up to a maximum value of 5 times the original dispersivity, $\alpha_0$. On a field scale dispersion is greater than on a laboratory scale, as additional dispersion is caused due to larger scale heterogeneities (Costanza-Robinson and Brusseau, 2006). This is assumed to reduce the relative effect of biofilm formation on the dispersivity parameter. The effect of biofilm formation on field scale heterogeneities is beyond the scope of this study. The relation used to model this is given in Equation 3.8.

$$\alpha = \alpha_0 + 4\alpha_0 \left( \frac{\phi_0 - \phi_p}{\phi_0 - \phi_{p,c}} \right)$$  \hspace{1cm} (3.8)

The effect is assumed to be the same for $\alpha_L$ and $\alpha_T$ in Continuum $P$. In Continuum $F$ dispersion is neglected due to the lower flow velocities.

### 3.3.4 Diffusion in the Biofilm

In the original model, diffusion in the biofilm is modelled using a molecular diffusion coefficient given by $D_{mol,c}^{\alpha, f} = \hat{D}_{mol,c}^{\alpha} S_{\alpha,f}^\tau_{\alpha,f} \phi_f$ for both the gas and the water phase. This accounts only for diffusion in the pores and neglects diffusion of solutes into the cell clusters. Stewart (1998) recommends a ratio between the effective diffusion coefficient in the entire biofilm relative to the molecular diffusion coefficient in water. The ratio is determined for different classes of solute. The effective diffusion coefficient $D_{eff,c}^{w}$ resulting from this is used for diffusion in the water phase. When the gas phase is present in the pores of the biofilm, molecular diffusion is determined as in the original model.

### 3.3.5 Fluid Viscosity

The viscosity change due to suspended solids is modelled using the formula by Einstein (1906) given in Equation 3.9. Suspended solids are assumed to be spherical particles.

$$\mu = \mu_0 \left( 1 + \frac{5}{2} \chi_w' \right)$$  \hspace{1cm} (3.9)
$X_{w}^{b}$ is the volume fraction of biomass in the water phase, and $\mu_0$ is the viscosity of the brine without suspended solids.

At mass fractions of biomass below 0.05, the Einstein formula gives the same change in viscosity as functions that take particle shape into account, according to [Rockhold et al. (2002)]. The increase in viscosity is up to 10% when the concentration of biomass is 0.05 kg/m$^3$.

### 3.3.6 Fluid Density

The density of water is not modified to account for suspended biomass, due to the small difference between the densities of microbial cells and water.
Chapter 4

Implementation of Electron Acceptor

Heterotrophic microorganisms consume both a substrate and an electron acceptor for growth. A variety of electron acceptors can be used. Oxygen is used preferentially by many species as it gives a high energy yield (Kim et al., 2004). The amount of oxygen, or other electron acceptors, available in deep aquifers is limited. This limitation is neglected in the original model, where only substrate is modelled as a requirement for growth. Adding a mass balance for the electron acceptor can give a more accurate representation of biofilm growth, as this will be limited when an electron acceptor is scarce. The main advantage is that it enables the simulation of separate injection of the electron acceptor and the substrate. Laboratory studies by Peyton (1996) and Thullner et al. (2002) indicate that the pulsed injection of a substrate and an electron acceptor leads to a more even distribution of biofilm throughout the porous medium, and that clogging near the injection point is reduced. When a pulsed injection strategy is applied it is important to have a model that can represent this.

4.1 Mass Balance

The electron acceptor that is implemented is oxygen. This can be present in both continua, and in both phases. The advantage of this is that it makes it possible to model the electron acceptor as a component of the CO$_2$ phase as well as of the water phase. Thus the electron acceptor can be injected during CO$_2$ injection. The mass balance equation for the electron acceptor is given by Equation 4.1

$$\frac{\partial (\phi_s S_{\alpha,\kappa} C_{\alpha,\kappa}^e)}{\partial t} + \nabla \cdot (C_{\alpha,\kappa}^e \mathbf{v}_{\alpha,\kappa}) - \nabla \cdot (D_{\alpha,\kappa}^e \nabla C_{\alpha,\kappa}^e) = q_{\alpha,\kappa}^e$$

$$\alpha \in \{w, n\}, \quad \kappa \in \{p, f\}$$

(4.1)
4.2 Transport

The electron acceptor is transported by advection, as represented by the second term of Equation 4.1, and dispersion, as represented by the third term. Hydrodynamic dispersion is implemented as discussed in Sections 2.2 and 3.3. This results in the following expressions for the dispersion tensors:

\[
\begin{align*}
D_{e}^{w,p} &= D_{\text{mech}}^{w,p} + D_{\text{mol,e}}^{w,p} S_{w,p} \phi_p \\
D_{e}^{g,p} &= D_{\text{mech}}^{g,p} + D_{\text{mol,e}}^{g,p} S_{g,p} \phi_p \\
D_{e}^{w,f} &= D_{\text{eff,e}}^{w} \\
D_{e}^{g,f} &= D_{\text{mol,e}}^{g,f} S_{g,f} \phi_f 
\end{align*}
\] (4.2)

For pulsed injections, the mixing of the substrate and the electron acceptor is driven by hydrodynamic dispersion. The validity of using the advection dispersion equation for reactive transport in porous media is questioned by Willingham et al. (2008). This approach may not accurately account for the mixing of reactants. There can be a significant variation in the concentration of reactants on the pore scale. According to Raje and Kapoor (2000), the consequence of this for the rates of reactions can be significant. These effects are not accounted for within the REV and are neglected in this study.

4.3 Distribution over the Phases

When both phases are present, the distribution of oxygen over the phases is assumed to be in thermodynamic equilibrium and given by Henry’s law. This assumption is used in similar models by Rockhold et al. (2005) and Ho (2006). Henry’s law is valid as long as the concentration of solute in the water phase is sufficiently dilute (Atkins, 2002) and is given in Equation 4.3.

\[
k_{He} = \frac{c_{w}^e}{p_{n}^e} \quad (4.3)
\]

Henry’s law relates the molar concentration of a component, \(c_{w}^e\) [mol/dm³], in the wetting phase to the partial pressure \(p_{n}^e\) of the component in the non-wetting phase. Using the ideal gas law, the Henry coefficient \(k_{He}\) can be converted to a dimensionless value \(k_{He}^{ce}\) that relates the concentration of the component in the two phases as shown in Equation 4.4 after Ho (2006).

\[
k_{He}^{ce} = C_{w}^e / C_{n}^e = k_{He}RT \quad (4.4)
\]

The units of \(k_{He}\) depend on the units in which pressure is expressed. \(R\) is the universal gas constant with units corresponding to those of \(k_{He}\). \(T\) is the absolute temperature in Kelvin.
Corrections are made for the temperature dependence of Henry’s constant using the enthalpy of solution after Sander (1999). The reduction of the solubility of oxygen due to salinity is accounted for using a reduction factor from Lewis (2006). The effect of dissolved oxygen on the density and viscosity of the water phase is neglected. The effect of dissolved CO$_2$ is more significant.

Exchange between the phases can be reduced due to the formation of biofilm on the interface between the phases according to Rockhold et al. (2005). In this case, as the gas is considered to be a biocide (Zhang et al., 2006), the accumulation of biofilm at the interface is neglected.

### 4.4 Distribution over the Continua

The concentration of the electron acceptor is equal in Continuum $P$ and Continuum $F$. This assumption implies that the biofilm is modelled fully penetrated by the electron acceptor.

Studies of biofilms utilising oxygen as the electron acceptor differ in the treatment of the penetration depth of oxygen in the biofilm. The penetration depth depends on the rate at which oxygen is consumed within the biofilm, and the rate at which it is supplied. Studies by Kim et al. (2004) and McLean et al. (2008) indicate oxygen is only present in the surface layer of the biofilm. Other authors including Semprini and McCarty (1991), Suidan et al. (1987) and Rockhold et al. (2005) consider biofilms to be fully penetrated by oxygen. The depth to which the electron acceptor penetrates the biofilm is important to determine whether the entire thickness of the biofilm contains active, growing, bacteria or only the surface layer. This affects the rate of biofilm accumulation and the maximum amount of biofilm that can form.

The assumption of full penetration of the biofilm is justified by considering the two extreme representations of biofilm structure, discussed in Chapter 3: a relatively uniform film and colonies plugging pores. The two situations are depicted in Figure 4.1.

#### 4.4.1 Case 1: Uniform Film

In this situation, the biofilm forms a layer over the grains, and does not bridge the pores, so that the fluid flows parallel to the biofilm. Transport of the electron acceptor into the biofilm is primarily due to diffusion according to Stoodley et al. (1997). Exchange of solutes, from Continuum $P$ to Continuum $F$, can be modeled by film theory. This uses the concept of a mass transfer boundary, a thin fluid film parallel to a solid surface, through which mass transfer takes place (Baehr, 1998). Studies have been carried out by De Beer et al. (1994) and Stoodley et al. (1997), to determine the geometry of the mass transfer boundary for oxygen above a biofilm for different flow velocities. For the flow velocities expected in the aquifer, the mass transfer boundary is parallel to
4.4 Distribution over the Continua

(a) Schematisation of biofilm growing as a film covering the grain surface. The forces driving fluid flow act from left to right. The direction of flow inside the pores is represented by arrows, flow is parallel to the grain surface.

(b) Schematisation of biofilm growing as a plug across pore throats. The forces driving fluid flow act from left to right and fluid is driven into the pores of the biofilm. The direction of flow inside the pores is represented by arrows.

Figure 4.1: Pore scale schematisation of two extreme biofilm growth structures, a relatively uniform film lining the pores, and plugs forming across pore throats.

the substratum, and not affected significantly by heterogeneities of the biofilm surface. Figure 4.2 illustrates flow of water past grains covered with biofilm.

Figure 4.2: Schematisation of flow past a grain surface covered by a relatively uniform biofilm. The arrows represent the velocity of water in the pore, this is lower near the biofilm surface. Flux is parallel to the grain surface.

Rittmann and McCarty (1980) model substrate penetration of a biofilm for the situation described above. They determine the maximum thickness at which a biofilm is fully penetrated by substrate, as a function of the concentration of substrate in the bulk of the water phase. For low substrate concentrations, in the order of $1 \times 10^{-3}$ kg/m$^3$, biofilm can be assumed to be penetrated up to a thickness of 50μm. Diffusion coefficients and utilisation rates of oxygen are in the same order of magnitude as those
for substrate, thus a similar penetration depth can be assumed. Pore entry sizes, for representative sandstone aquifers, are found in the order of up to 90μm (Bloomfield et al., 2001). Thus, uniform biofilm only lining the pores can be assumed to be less than 30μm thick. These films can be assumed to be fully penetrated by oxygen, unless the concentration of oxygen becomes very low. When there is little oxygen present in the REV, the biofilm will consume all of it. Growth is then limited by a lack of total mass of oxygen, not a lack of penetration into the biofilm. For modelling on the macroscale it is irrelevant at what depth in the biofilm this growth takes place, so the assumption of full penetration appears reasonable for uniform biofilms.

4.4.2 Case 2: Biofilm Plugs

In the case where biofilm colonies plug the pores, the forces driving flow act across the biofilm. The electron acceptor is transported into the pores of the biofilm by advection. Biofilm porosity is in the range of 80% (Zhang and Bishop, 1994a) (Lewandowski, 2000), so the distance that the electron acceptor has to diffuse through the cell clusters is relatively low. If few pores in the REV are plugged by the biofilm, the concentration of oxygen in Continuum $F$ can be considered equal to the concentration in Continuum $P$. When the total biomass volume fraction is very high, fluid in Continuum $F$ can become depleted of oxygen faster than fluid in Continuum $P$. In this case the porosity, and thus the total volume of fluid, in Continuum $P$ is low and the total mass of oxygen present is low. Growth is then limited by the total mass of oxygen and the distribution of this in the biofilm is not relevant to a macroscale approach.

4.5 Consumption by Biomass

The exchange term $q_{w,k}$ accounts for consumption of the electron acceptor by the biomass for respiration, and external sources of electron acceptor. The carbon source used for respiration can be a substrate, in the case of growth, or cell components, in the case of endogenous decay.

4.5.1 Biomass Growth

The growth rate for biomass consuming two components is generally modelled using double Monod kinetics (Molz et al., 1986; Rockhold et al., 2005; Semprini and McCarty, 1991). The growth rate Equation 1.20, from the original model, is modified to give the following equation for the biofilm growth rate:

$$
\mu_k = k_\mu Y \frac{C_{w,k}^s}{K_s + C_{w,k}^s} \frac{C_{w,k}^e}{K_e + C_{w,k}^e}
$$
4.5 Consumption by Biomass

$C_{w,\kappa}$ is the concentration [kg/m$^3$] of the electron acceptor in water in Continuum $\kappa$. $K_e$ is the Monod half saturation constant for the electron acceptor, which is defined similar to $K_s$ in Section 1.4. From the stoichiometric equation for the growth of biomass, the ratio of the mass of the electron acceptor consumed to the mass of the substrate consumed can be determined, this is given by the factor $R$. The rate of electron acceptor consumption for growth, is given in Equation 4.6, where $r_{g,\kappa}$ is given by Equation 1.19 in Section 1.4.

$$q_{\kappa}^{e,\text{growth}} = -R \frac{r_{g,\kappa}}{Y}$$  \hfill (4.6)

4.5.2 Biomass Decay

An electron acceptor is also consumed by cells when they respire on intracellular material. This process is often included in models accounting for an electron acceptor (Borden and Bedient, 1986; Molz et al., 1986; Rockhold et al., 2005; Semprini and McCarty, 1991). When endogenous decay is dependent on the presence of an electron acceptor, the biofilm will remain in place indefinitely when the electron acceptor has been depleted. Models where an electron acceptor is required for endogenous decay generally have a water phase that naturally contains dissolved oxygen. A situation of zero decay does not occur, and Semprini and McCarty (1991) conclude their model matches field observations quite well. Including the consumption for endogenous decay in the mass balance improves the accuracy of modelling the mass of the electron acceptor.

Measurements by Cunningham et al. (2003) show that the permeability reduction achieved by an in situ bio-barrier can remain relatively constant for up to 10 months after nutrient injections are stopped. It is suggested that, once the biofilm is established, it remains intact in the pore space, whilst the bacterial cells revert to a semi-starved state in the absence of a carbon source and an electron acceptor. This supports the suggestion biofilm remains in place if it is not consumed by endogenous decay. However, increases in permeability are observed, indicating there is deterioration of the biofilm (Cunningham et al., 2003).

In this model there is no distinction between active and inactive biomass. Endogenous decay, in this model, is used to account for the consumption of cellular material for energy, which requires an electron acceptor, as well as other reductions in the activity of the cells that are not caused by CO$_2$. (Reductions in bacterial activity due to CO$_2$ are accounted for in separate decay terms as discussed in Sections 1.4 and 5.3). Predation by protozoa is one other factor that can cause deactivation of bacterial cells in natural environments, according to van Loosdrecht and Henze (1999). Making the entire endogenous decay term dependent on the electron acceptor, implies there is no other reduction in bacterial activity. This is likely to overestimate the amount of active biomass when the electron acceptor is scarce.
The possibility of having endogenous decay dependent on the electron acceptor, is implemented using single Monod kinetics, as done by Semprini and McCarty (1991) and Rockhold et al. (2005). The endogenous decay rate coefficient, \( b_{0,\kappa} \), that is dependent on the concentration of the electron acceptor is given in Equation 4.7. In Chapter 6 the effects of using constant endogenous decay, and endogenous decay according to Equation 4.7 is investigated.

\[
b_{0,\kappa} = b_e \frac{C_{\alpha,\kappa}^e}{K_e + C_{\alpha,\kappa}^e} \quad (4.7)
\]

The rate of endogenous decay in the two continua is given by Equation 4.8.

\[
r_{0,b,p} = b_{0,p} \phi_p S_{w,p} C_w^b \\
r_{0,b,f} = b_{0,f} (\phi_f / \varepsilon) q_b 
\quad (4.8)
\]

The amount of an electron acceptor that is required for endogenous decay is determined from the stoichiometric formula for biomass and from the fraction of the cells that is biodegradable. The rate of electron acceptor consumption due to endogenous decay is given by in Equation 4.9.

\[
q_{e,\text{decay}} = -r_{0,b,\kappa} d_c f_d 
\quad (4.9)
\]

\( d_c \) is the mass of oxygen required per mass of cells decayed, \( f_d \) is the fraction of biomass that is biodegradable (Semprini and McCarty, 1991). The total consumption of the electron acceptor by the biomass is given by Equation 4.10 \( \hat{q}_{\alpha,\kappa}^e \) accounts for external sources of electron acceptor, which can be in both phases.

\[
q_{\alpha,\kappa}^e = q_{\kappa}^{e,\text{growth}} + q_{\kappa}^{e,\text{decay}} + \hat{q}_{\alpha,\kappa}^e \quad (4.10)
\]
Chapter 5

Lysis due to Carbon Dioxide

In order to adequately model lysis of microorganisms by CO$_2$, it is important to assess the mechanisms that are responsible for causing lysis. In the original model, there is no lysis in absence of supercritical CO$_2$, as dissolution of CO$_2$ is not accounted for. In this work, it is suggested that high concentrations of CO$_2$ in water can also cause lysis. Much research is carried out to investigate the use of CO$_2$ under pressure as a sterilisation agent for medical purposes and food sterilisation. A discussion of several experiments is presented to investigate plausible mechanisms for cell deactivation. This is important to assess whether processes causing deactivation in experiments also play a role in the aquifer, if suspended and attached bacteria are affected in the same way, and whether dissolved CO$_2$ or only supercritical and gaseous CO$_2$ can cause lysis.

5.1 Lysis of Suspended Cells

Experiments investigating microbial deactivation due to high pressure CO$_2$ concentrate on different factors to optimise the sterilisation of bacterial cells. An extensive experimental review is presented by Zhang et al. (2006), where experiments covering 22 microbial species are evaluated. Despite differences in the deactivation rates and treatment times, 20 species were completely deactivated by treatment with CO$_2$ under high pressure.

In the following section, the causes for lysis by CO$_2$ suggested by various authors are examined. The proposed mechanisms of deactivation generally fall into two categories, mechanical and physiological damage to the cells. The relative importance of these mechanisms may vary for different types of organism according to Mitchell et al. (2008). Experiments are mainly conducted on cells in suspension; there are few investigations regarding the inactivation of cells in a biofilm. Based on experiments by Mitchell et al. (2008) and Mun et al. (2009), the effect of CO$_2$ on microbial cells in a biofilm is compared to the effect on suspended cells in Section 5.2.
5.1 Lysis of Suspended Cells

5.1.1 Presence of Water

In a review, considering 50 articles on microbial deactivation due to high pressure CO\textsubscript{2}, Spilimbergo and Bertucco (2003) conclude that microbial deactivation is related to the concentration of dissolved CO\textsubscript{2}. Various experiments for suspended cells indicate deactivation occurs in two stages. The first one has a low deactivation rate, which is attributed by Lin et al. (1992) to dissolution of CO\textsubscript{2} in water, and subsequent diffusion through the cell membranes into the cells. The second stages has a higher deactivation rate. This stage initiates when the concentration of CO\textsubscript{2} inside the cells reaches a critical level where it causes cell deactivation (Lin et al., 1992; Wu et al., 2007).

The presence of water appears to be a requirement for the deactivation of microbial cells by CO\textsubscript{2}. Drying a sample, to reduce the water content to 6%, results in a greatly lowered deactivation of microbial cells in experiments by Debs-Louka et al. (1999). Kamihira et al. (1987) also report that cells with a water content below 10% could not be sterilised by CO\textsubscript{2}. Lin et al. (1994) suggest that the presence of water causes cell walls to swell, making them more permeable to CO\textsubscript{2}. Spilimbergo and Bertucco (2003) suggest interference of dissolved CO\textsubscript{2} with the cell walls changes the structure of the cell membrane, which increases its permeability to CO\textsubscript{2}. Without the presence of CO\textsubscript{2} inside the cells, deactivation might not take place.

5.1.2 High Pressure

To determine the effect of pressure alone on microbial cells, they are subjected to high pressures of water, nitrogen and air. Lin et al. (1992) find that 90% of the cells survive treatment with nitrogen at a pressure of 12 MPa. CO\textsubscript{2} treatment at the same pressure results in a 7 log\textsubscript{10} reduction in the number of viable cells. Similar results are reported from other experiments, deactivation rates due to high pressure alone are significantly lower than those due to exposure to high pressure CO\textsubscript{2}. This leads many authors to conclude that high pressure is not the principal cause of deactivation (Debs-Louka et al., 1999; Haas et al., 1989; Lin et al., 1992). Zhang et al. (2006) suggest hydrostatic pressures greater than 100 MPa are required to deactivate bacteria, based on data from Cheftel (1995). This confirms that biofilm can grow under the pressure conditions in this model, and that lysis due to CO\textsubscript{2} probably has another cause.

Wu et al. (2007) suggest cells rupture when the pressure is released rapidly after treatment. If this is the main cause of lysis, it will not take place in situations that are modelled in this work. Lin et al. (1992) report that cycles of pressure release, during nitrogen treatment of yeast cells at 12 MPa, do not affect deactivation rates. Foster et al. (1962) find depressurisation of nitrogen, at pressures of 12 MPa, causes cell rupture in up to 58% of the microbial cells. This is significantly less than the reduction of living cells during CO\textsubscript{2} treatment. These findings suggest depressurisation may account for some decrease in microbial activity, but it is not the principal cause of the efficiency of
5.1 Lysis of Suspended Cells

CO\textsubscript{2} treatment.

5.1.3 State of Aggregation of CO\textsubscript{2}

If CO\textsubscript{2} only causes lysis in the supercritical state of aggregation, dissolved CO\textsubscript{2} will not have an effect on the decay of microbial cells. Supercritical CO\textsubscript{2} is a good solvent that is used in various extraction processes (Mukhopadhyay, 2009). Its ability to extract intracellular material, is suggested to be a cause of its efficiency as a biocide by Kamihira \textit{et al} (1987) and Zhang \textit{et al} (2006). When CO\textsubscript{2} is not in the supercritical state its extraction power is significantly reduced. Experiments, carried out with CO\textsubscript{2} that is not in the critical state, still show deactivation of microorganisms but at a lower rate (Debs-Louka \textit{et al}, 1999; Haas \textit{et al}, 1989; Lin \textit{et al}, 1992, 1994; Kamihira \textit{et al}, 1987). Lin \textit{et al} (1992) suggest that the mechanisms for deactivation are the same for supercritical CO\textsubscript{2} and gaseous or liquid CO\textsubscript{2}. This implies that extraction of intracellular material is not the only mechanism by which cells are deactivated by CO\textsubscript{2}, though it may play a role in increasing the rate of deactivation.

5.1.4 Reduced Extracellular pH

Hydration of CO\textsubscript{2}, and dissolution of the resulting carbonic acid lowers the pH of the solution. In the aquifer, the different salts may act as a buffer limiting the pH change. Changes in pH are not included in this model, but should be if this is the predominant mechanism by which cells are deactivated.

The reduction of the pH of a solution by other acids is found not to cause deactivation of microbial cells to the extent that treatment with CO\textsubscript{2} does (Debs-Louka \textit{et al}, 1999). The other acids tested can not penetrate the cell walls as easily, and therefore do not have the same deactivating effect (Debs-Louka \textit{et al}, 1999; Haas \textit{et al}, 1989). Bacteria that thrive under conditions of a low pH are still deactivated by treatment with supercritical CO\textsubscript{2} in experiments by Haas \textit{et al} (1989). Wu \textit{et al} (2007) investigate the effect of CO\textsubscript{2} treatment without a reduction of the pH, by adding a NaHCO\textsubscript{3} buffer to the solution of bacterial cells. This gives a slight increase in the deactivation rate. They suggest that due to the presence of the ions formed by dissociation of carbonic acid, HCO\textsuperscript{−}\textsubscript{3} and CO\textsubscript{3}\textsuperscript{2−}, less hydrated CO\textsubscript{2} dissociates. This leaves more molecular CO\textsubscript{2} in the solution, and the penetration of cell membranes by molecular CO\textsubscript{2} is more rapid.

5.1.5 Reduced Intracellular pH

Molecular CO\textsubscript{2} can penetrate cell membranes and enters the microbial cells (Spilimbergo and Bertucco, 2003). In the cells, the dissociation of carbonic acid lowers the intracellular pH. This is likely to be an important cause of cell deactivation.
Lowering the pH inside the cells is reported to cause the deactivation of certain enzymes (Haas et al., 1989; Kamihira et al., 1987; Mitchell et al., 2008; Wu et al., 2007). It is also suggested that a lowered pH interferes with metabolic processes of the cell (Haas et al., 1989; Lin et al., 1992; Mitchell et al., 2008).

### 5.1.6 Type of Microorganism

The resistance of microorganisms to treatment with CO\textsubscript{2} varies significantly for different species (Haas et al., 1989; Kamihira et al., 1987; Wu et al., 2007). A review by Zhang et al. (2006), of experiments conducted with 12 species of gram positive and 10 species of gram negative bacteria, shows that gram positive organisms are generally more resistant to treatment. Gram positive cells have cell membranes that are thicker, stronger and less permeable than gram negative cells, according to Zhang et al. (2006). Experiments by Wu et al. (2007), on different species of gram negative and gram positive cells, show a similar amount of deactivation for both types. They suggest that though gram positive cells have a greater amount of polymer in the cell walls, this does not contain components that prevent CO\textsubscript{2} from entering the cells, therefore, no significant difference in deactivation rate is expected.

### 5.1.7 Summary

Deactivation rates are variable between species, and several mechanisms may play a role in causing the deactivation of bacteria. From the experiments cited above, it appears that penetration of cell membrane by CO\textsubscript{2}, and the subsequent physiological disruption, is considered an acceptable primary mechanism of deactivation. The precise effects of CO\textsubscript{2} inside the cells that cause lysis are still uncertain, it may be the deactivation of particular enzymes, disruptions of metabolic pathways, extraction of intracellular material or a combination of these. When the first two are the case, it is not required for the cells to be in contact with supercritical CO\textsubscript{2}, dissolved CO\textsubscript{2} will also cause lysis. For extraction of intracellular material, contact with supercritical CO\textsubscript{2} is required.

### 5.2 Lysis of Attached Cells

Suspended cells are found to be deactivated to a greater extent than cells in biofilms by Mitchell et al. (2008) and Mun et al. (2009). Mitchell et al. (2008) report a 0.05 log\textsubscript{10} reduction of viable cells in a biofilm, after a 19 minute exposure to supercritical CO\textsubscript{2}, compared to a 3 log\textsubscript{10} reduction in suspended cells. They conclude that EPS plays a significant role in the protection of cells in the biofilm.

It is suggested by Mitchell et al. (2008) that supercritical CO\textsubscript{2} penetrates the biofilm and sorbs to the EPS, causing swelling and plasticisation of the EPS. They report noticeable swelling of biofilm and sand matrix as a result of treatment with supercritical CO\textsubscript{2}. Studies by Liu and Tomasko (2007) and Uzer et al. (2006) indicate
5.3 Modelling Lysis due to CO$_2$

Interaction with the EPS may slow the penetration of CO$_2$ into the biofilm, and reduce the penetration of CO$_2$ into microbial cells.

Mun et al. (2009) report that cells in biofilms, which are immersed in water during treatment with supercritical CO$_2$, are lysed at a lower rate than biofilm in direct contact with supercritical CO$_2$. During the same length of exposure, biofilms in contact with supercritical CO$_2$ show a 6 log$_{10}$ reduction in viable cells, whereas biofilms immersed in water show a 1.5 log$_{10}$ reduction. It appears that more interaction between the supercritical CO$_2$ and the biofilm leads to a higher deactivation rate of microbial cells.

5.3 Modelling Lysis due to CO$_2$

Based on the experiments discussed in the previous sections, a term is included in the decay rate expression to account for increased lysis due to dissolved CO$_2$. This is shown in Equation 5.1, where $b_{c2,\kappa}$ is a function of the mass fraction of dissolved CO$_2$, $X_{w,\kappa}$.

$$
b_{\kappa} = b_0 + b_{c1,\kappa} + b_{c2,\kappa}$$

$$
b_{c2,\kappa} = c_2 f(X_{w,\kappa}^{CO_2})$$

(5.1)

In order to have a significant effect on bacterial cells, a minimum mass fraction of CO$_2$ should be present, from this point onwards the effect increases rapidly. The function $f(X_{w,\kappa}^{CO_2})$ and the parameter $X_{tox,\kappa}$ is introduced to represent this as shown in Equation 5.2. As the mass fraction of CO$_2$ approaches $X_{tox,\kappa}$ the value of $f(X_{w,\kappa}^{CO_2})$ rises sharply from 0 to 1 as shown in Figure 5.1.

$$
x_{e,\kappa} = \begin{cases} 
X_{w,\kappa}^{CO_2} / X_{tox,\kappa} & \text{if } X_{w,\kappa}^{CO_2} \leq X_{tox,\kappa} \\
1 & \text{if } X_{w,\kappa}^{CO_2} \geq X_{tox,\kappa} 
\end{cases}
$$

$$
f(X_{w,\kappa}^{CO_2}) = \frac{1}{1 + \left(\frac{1 - x_{e,\kappa}^4}{x_{e,\kappa}^4}\right)}$$

(5.2)

The effect of CO$_2$ is greater for suspended cells than for cells inside the biofilm. This can be accounted for by a lower threshold $X_{tox,\kappa}$ in Continuum $P$ than in Continuum $F$. Supercritical CO$_2$ has a greater effect on cell lysis than dissolved CO$_2$ as shown by Kamihira et al. (1987), Lin et al. (1992) and others. When both dissolved and free-phase CO$_2$ are present in Continuum $F$, lysis due to the supercritical CO$_2$ will dominate. Including the effect of dissolved CO$_2$ can be important, as this can penetrate the biofilm much faster than free-phase CO$_2$. Continuum $F$ has a higher entry pressure than Continuum $P$, thus the saturation of free-phase CO$_2$ in Continuum $P$ must be relatively high before the free-phase CO$_2$ enters Continuum $F$ (refer to Figure 1.6 in Section 1.4).
Figure 5.1: The factor $f(X_{\text{CO}_2})$ scales the effect of dissolved CO$_2$ as a biocide. The effect is low at low concentrations and rises sharply as the critical value $X_{\text{tox,}_R}$ is approached, in this graph $X_{\text{tox,}_R} = 0.04$.

The difference in potency as a biocide for supercritical and dissolved CO$_2$ is reflected in the ratio of the values of the parameters $c_{c1}$ and $c_{c2}$. These values give the maximum rate of lysis due to supercritical and dissolved CO$_2$ respectively. Data from [Mun et al. (2009)], indicate the effect of dissolved CO$_2$ is 4 to 5 orders of magnitude smaller than that of the free-phase CO$_2$ in contact with the biofilm. The effect of different values of $c_{c2}$ in the range of 3 to 5 orders of magnitude less than $c_{c1}$ is investigated in Chapter 6.
Chapter 6

Effect of Model Modifications

The effects of modifications made to the original model are investigated in this section. Firstly, the modifications that were made to improve the representation of the accumulation of biofilm are considered. A reference simulation for pulsed injection, of a substrate and an electron acceptor is presented, and the pattern of biofilm accumulation is discussed. Simulations are compared to this to investigate the effects of: adding dispersion to the model, variations in endogenous decay rate parameters, different injection strategies, and lysis due to dissolved CO$_2$. In the second section of this chapter, the adaptations that are made to the hydraulic parameters affecting two-phase flow are considered.

6.1 Parameters Affecting Biofilm Development

6.1.1 Reference Simulation

The purpose of the reference simulation is to analyse the effect of a particular injection strategy on the mass accumulation of biofilm. In the reference simulation all modifications made to the model are included, except for the dependence of the decay rate on the electron acceptor. Decay is modelled as is done in the original model. Subsequent simulations are done where these modifications are excluded one at a time. Then simulations with different injection strategies are performed. These are compared to the reference simulation to gain an understanding of the effect of different parameters, and of several pulsed injection strategies.

6.1.1.1 Model Domain and Parameters

The region of interest is the area near the injection point. The simulation domain is two-dimensional, with a height and width of 10 m respectively. The mesh has a uniform spacing of 1 m in both directions, as shown in Figure 6.1. The model is isothermal with a temperature of 40°C, and the initial pressure is $1 \times 10^7$ Pa, which is above the critical point of CO$_2$. The two phases that can be present in these
simulations are the water phase and the CO\textsubscript{2} phase, water is the wetting phase. Gravity is not included for these simulations.

Figure 6.1: The domain is two-dimensional with a length and width of 10 m and the mesh has an even spacing of 1 m.

The model contains many empirical parameters related to bacterial activity. Values for these are obtained from model verification by Ebigbo (2009), as well as from literature, as there is no parameter set that contains all relevant parameters. Semprini and McCarty (1991) note that rate coefficients for growth and decay vary for different species of bacteria using the same substrate, and may even change for the same species in the course of time. To gain a qualitative understanding of the model response to different modifications, the use of representative literature values is sufficient. Biological parameters used in the simulations are given in Table 6.1. Biomass attachment and detachment parameters used are taken from Ebigbo (2009) and listed in Table 6.2.

The formation properties and the hydraulic properties of the biofilm are given in Table 6.3. Formation properties are based on values from sand column experiments described by Taylor and Jaffe (1990). Fluid properties are given in Table 6.4. The parameters used to determine relative permeability and saturation are given in Table 6.5. The diffusion and dispersion coefficients used for hydrodynamic dispersivity are given in Table 6.6.

At the start of the simulation, there is no CO\textsubscript{2} or substrate in the domain, the concentration of the electron acceptor is $1 \times 10^{-4}$ kg/m$^3$, and a very small amount of biofilm and microbial cells are present (the volume fraction of biofilm, $\phi_f/\varepsilon = 0.0001$).

The north and south boundaries are no flow boundaries. The west boundary is where injection takes place, water is injected at a rate of 0.002 kg/(m$^2$s) over the entire length of the boundary throughout the simulation. A small amount of oxygen, in equilibrium with atmospheric pressure, is dissolved in the injected water. The east boundary is a Dirichlet boundary (except for the variable representing the volume fraction of biofilm, which is Neumann with zero flow). The concentrations of
### Table 6.1: Biological parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum substrate utilisation rate $k_{\mu}$</td>
<td>$8.91 \times 10^{-5} \text{s}^{-1}$</td>
<td>(Ebigbo, 2009)</td>
</tr>
<tr>
<td>Monod half-saturation coefficient electron acceptor $K_e$</td>
<td>$2.0 \times 10^{-3} \text{kg/m}^3$</td>
<td>(Semprini and McCarty, 1991)</td>
</tr>
<tr>
<td>Monod half-saturation coefficient substrate $K_s$</td>
<td>$1.5 \times 10^{-3} \text{kg/m}^3$</td>
<td>(Semprini and McCarty, 1991)</td>
</tr>
<tr>
<td>Yield coefficient $Y$</td>
<td>0.5 kg/kg</td>
<td>(Semprini and McCarty, 1991)</td>
</tr>
<tr>
<td>Ratio electron acceptor to substrate requirement of biomass $R$</td>
<td>0.5 kg/kg</td>
<td>(Semprini and McCarty, 1991)</td>
</tr>
<tr>
<td>Endogenous decay rate $b_0$</td>
<td>$3.18 \times 10^{-7} \text{s}^{-1}$</td>
<td>(Ebigbo, 2009)</td>
</tr>
<tr>
<td>Decay rate parameter $c_{c,1}$</td>
<td>$8.7 \times 10^{-4} \text{s}^{-1}$</td>
<td>(Ebigbo, 2009)</td>
</tr>
<tr>
<td>Decay rate parameter $n_c$</td>
<td>3</td>
<td>(Ebigbo, 2009)</td>
</tr>
<tr>
<td>Biofilm density $\rho_b$</td>
<td>3 kg/m$^3$</td>
<td>(Ebigbo, 2009)</td>
</tr>
</tbody>
</table>

### Table 6.2: Biomass attachment and detachment parameters after Ebigbo (2009).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attachment rate parameter $c_{a,1}$</td>
<td>$7.40 \times 10^{-3}$</td>
</tr>
<tr>
<td>Attachment rate parameter $c_{a,2}$</td>
<td>$7.88 \times 10^{-2}$</td>
</tr>
<tr>
<td>Detachment rate parameter $c_{d,1}$</td>
<td>$2.9 \times 10^{-8}$</td>
</tr>
<tr>
<td>Detachment rate parameter $\tilde{c}_{d,2}$</td>
<td>6</td>
</tr>
</tbody>
</table>

### Table 6.3: Properties of the porous medium and the biofilm.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permeability</td>
<td>$1.415 \times 10^{-10} \text{m}^2$</td>
</tr>
<tr>
<td>Porosity</td>
<td>0.347</td>
</tr>
<tr>
<td>Specific surface of sand $M$</td>
<td>$4.85 \times 10^3 \text{m}^{-1}$</td>
</tr>
<tr>
<td>Permeability parameter $\phi_{p,c}/\phi_0$</td>
<td>0.6</td>
</tr>
<tr>
<td>Permeability parameter $n_k$</td>
<td>3</td>
</tr>
<tr>
<td>Minimum permeability $K_{\text{min}}$</td>
<td>$0.01 \cdot K_0$</td>
</tr>
<tr>
<td>Biofilm porosity $\varepsilon$</td>
<td>0.8</td>
</tr>
</tbody>
</table>
6.1 Parameters Affecting Biofilm Development

Table 6.4: Fluid properties.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brine salinity</td>
<td>0.05 kg/kg</td>
<td></td>
</tr>
<tr>
<td>Brine density $\rho_w$</td>
<td>$f(p, T, S)$</td>
<td>(Batzle and Wang, 1992)</td>
</tr>
<tr>
<td>Brine viscosity $\mu_w$</td>
<td>$f(p, T, S, C^b)$</td>
<td>(Batzle and Wang, 1992; Einstein, 1906)</td>
</tr>
<tr>
<td>CO$_2$ density $\rho_n$</td>
<td>$f(p, T)$</td>
<td>(Span and Wagner, 1996)</td>
</tr>
<tr>
<td>CO$_2$ viscosity $\mu_n$</td>
<td>$f(p, T)$</td>
<td>(Fenghour et al., 1998)</td>
</tr>
</tbody>
</table>

Table 6.5: Parameters for capillary pressure and relative permeability.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pore-size distribution indices</td>
<td></td>
</tr>
<tr>
<td>$\lambda_p$</td>
<td>1.0</td>
</tr>
<tr>
<td>$\lambda'_p$</td>
<td>3.0</td>
</tr>
<tr>
<td>$\lambda_f$</td>
<td>0.1</td>
</tr>
<tr>
<td>$l$</td>
<td>1/3</td>
</tr>
<tr>
<td>Entry pressures</td>
<td></td>
</tr>
<tr>
<td>$p_{d,p}$</td>
<td>0.1 bar</td>
</tr>
<tr>
<td>$p_{d,f}$</td>
<td>0.01 bar</td>
</tr>
<tr>
<td>Residual saturations</td>
<td></td>
</tr>
<tr>
<td>$S_{wr,p}$</td>
<td>0.1</td>
</tr>
<tr>
<td>$S_{wr,f}$</td>
<td>0.3</td>
</tr>
<tr>
<td>$S_{nr,p}$</td>
<td>0.05</td>
</tr>
<tr>
<td>$S_{nr,f}$</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 6.6: Parameters for hydrodynamic dispersion

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular diffusion coefficients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\hat{D}_{mol,e}$</td>
<td>$2.3 \times 10^{-9}$ m$^2$/s</td>
<td>(Han and Bartels, 1996)</td>
</tr>
<tr>
<td>$\hat{D}_{mol,s}$</td>
<td>$6.7 \times 10^{-10}$ m$^2$/s</td>
<td>(Longsworth, 1955)</td>
</tr>
<tr>
<td>$\hat{D}_{mol,b}$</td>
<td>$6.7 \times 10^{-10}$ m$^2$/s</td>
<td></td>
</tr>
<tr>
<td>$\hat{D}_{mol,CO_2}$</td>
<td>$2.2 \times 10^{-10}$ m$^2$/s</td>
<td>(Tamimi et al., 1994)</td>
</tr>
<tr>
<td>$\hat{D}_{g,e}$</td>
<td>$1.0 \times 10^{-5}$ m$^2$/s</td>
<td>(Oldenburg, 2006)</td>
</tr>
<tr>
<td>$\hat{D}_{g,w}$</td>
<td>$1.0 \times 10^{-5}$ m$^2$/s</td>
<td>(Oldenburg, 2006)</td>
</tr>
<tr>
<td>Ratios for effective diffusion in biofilm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\left( \frac{D_{e,f,s}}{D_{e,w,mol}} \right)$</td>
<td>0.45</td>
<td>(Stewart, 1998)</td>
</tr>
<tr>
<td>$\left( \frac{D_{e,f,CO_2}}{D_{e,w,mol,CO_2}} \right)$</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>$\left( \frac{D_{s,f,s}}{D_{s,w,mol}} \right)$</td>
<td>0.25</td>
<td></td>
</tr>
</tbody>
</table>
the substrate, the electron acceptor and the suspended biomass at this boundary are $1 \times 10^{-9}$, ensuring results are not affected by these components entering the domain through the boundary.

The injection strategy is a 5 day pulse of solution containing the substrate, followed by a 5 day break in which only water is injected, then a 5 day pulse of solution containing the electron acceptor, followed by another 5 day break where only water is injected. This pattern is repeated for 7 injection cycles. The concentrations of both the substrate and the electron acceptor in the injected solution are $0.5 \text{ kg/m}^3$. Microbial cells are injected with the first pulse of the electron acceptor with a concentration of $0.005 \text{ kg/m}^3$ for 5 days.

6.1.1.2 Results

The rate at which the electron acceptor is consumed is twice as high as the rate at which the substrate is consumed (as indicated by parameter $R$ in Table 6.1). A motivation to inject excess substrate during the growth phase, is that the electron acceptor can be injected also in the CO$_2$ phase. This could potentially stimulate growth during CO$_2$ injection and help maintain the integrity of the biofilm barrier. This is investigated using simulations further on in this section.

The effect of pulsed injection on the mass of biofilm formed throughout the simulation is shown in the right image of Figure 6.2. The curve represents the total mass of attached biofilm (suspended cells are not included in this). The bars indicate pulses where the substrate and the electron acceptor are injected. The left image of Figure 6.2 shows the total mass of the substrate and the electron acceptor present in the domain.

When the first pulse of the substrate is injected, there is little growth, as there is only a minimal amount of the electron acceptor and biomass in the aquifer. As there is no consumption of the substrate, the first peak in the curve showing the total mass of substrate is higher than following peaks.

The electron acceptor is injected in combination with microbial cells. As soon as the electron acceptor is injected, it mixes with the substrate and is consumed by biomass. Due to this, the peak in the curve for the mass of the electron acceptor is lower than the one for substrate. The total increase in mass of biofilm is greatest in the first cycle, due to the attachment of injected microbial cells in addition to the regular growth of the biomass.

In the break after the injection of the pulse of the electron acceptor, the growth rate gradually levels off as the concentration of the substrate in the zone where the biofilm grows decreases. In this period, the total mass of biofilm is not decreasing indicating growth still compensates for endogenous decay.

When the next pulse of substrate is injected, the growth rate increases sharply. The curve representing the mass of the electron acceptor has an inflection point at this time,
Figure 6.2: The substrate and the electron acceptor are injected separately for 5 days, with 5 day breaks between each injected pulse, for 7 injection cycles. The same mass of the components is injected, but the electron acceptor is consumed at a higher rate. The total mass of biofilm present after each injection cycle tends towards a constant level. When injection is stopped endogenous decay causes a reduction in the mass of biofilm.
indicating the rate of consumption of the electron acceptor increases. During substrate injection, the electron acceptor becomes scarce within 1 day. As there is little electron acceptor in the zone containing biofilm, little substrate is consumed and the substrate accumulates. A decrease in total mass of biofilm indicates the decay rate exceeds the growth rate during this period. The next pulse of the electron acceptor induces a new high growth rate, which declines as the substrate concentration falls. Biofilm decay after the substrate pulse is greater than after the pulse of the electron acceptor. The mass of electron acceptor falls to zero during each injection cycle, but some of the substrate always remains in the domain. There is, however, no steady accumulation of the substrate in the domain. Due to the continuous injection of water, the substrate leaves the domain over the eastern boundary. In order to maintain an excess amount of the substrate in the biofilm, for possible growth by co-injection of an electron acceptor in the CO$_2$, the flow of water should be stopped immediately after the substrate injection.

The peak where the most biomass is present increases with successive injection cycles, but it appears to approach a constant value. It is noted in a similar model by Semprini and McCarty [1991] that for a given pulsing strategy, the mass of biofilm reaches a steady state level after a sufficiently long time. The endogenous decay rate depends on the amount of biofilm present, and thus increases as more biofilm accumulates. The growth rate can not increase beyond that which is possible due to scarcity the substrate or the electron acceptor. Thus, an equilibrium state is reached, where the total growth equals the total decay during one injection cycle.

### 6.1.2 Dispersion

Two simulations are performed in this section. The aim of the first simulation is to determine if the addition of mechanical dispersion to the model has a noticeable effect on biofilm accumulation. Accounting for mechanical dispersion, in addition to molecular diffusion, increases the spreading of a pulse of dissolved components. This is expected to result in an increased mixing of the electron acceptor and the substrate, which should be reflected by a greater accumulation of biofilm.

A simulation is performed that is identical to the reference simulation, but with the mechanical dispersion term set to zero. The total mass of biofilm that accumulates is plotted for this simulation and the reference simulation in Figure 6.3.

The highest peaks in the curve representing biofilm mass when dispersion is accounted for are slightly higher than the ones in the curve where dispersion is not accounted for. This indicates dispersion results in an increased mixing of solutes, and an increase in the total mass of biofilm that is formed. The peaks occur slightly earlier when dispersion takes place, indicating mixing is also faster when mechanical dispersion is included.
During the phase when the total mass of biofilm decreases, (the intervals when the electron acceptor is scarce, but the substrate is present, as discussed for the reference simulation), the curve without dispersion is marginally higher than the curve with dispersion. This may indicate that without dispersion, more of the electron acceptor is present in the biofilm at this period in the injection cycle. The biofilm growth enabled by this compensates, to some extent, the endogenous decay of biofilm. Thus the effect of increased dispersion, which enables more and faster growth, is offset by a more rapid depletion of the electron acceptor. The difference between the two curves tends toward a constant value, as a steady state level of biofilm mass is approached.

A second simulation is carried out to assess the effect of modelling an increase in the value of the dispersion coefficient, $\alpha$, with the volume fraction of biofilm, as discussed in Chapter 3. It is expected that an increase in $\alpha$ causes an increase in mixing in the area where biofilm is present. This should lead to an greater total mass of biofilm, compared to a situation where $\alpha$ is constant.

The simulation is performed with the same domain, boundary conditions, and parameters as the reference simulation. In order to have a noticeable effect on dispersion the volume fraction of biofilm must be relatively high. Therefore, the decay rate is reduced by a factor 10, allowing for a greater total mass of biofilm to accumulate. The injection strategy is the same as for the reference simulation, except that the concentrations of the substrate and the electron acceptor are doubled, and only 4 cycles are injected. The simulation is performed twice, once where $\alpha$ is constant and once where $\alpha$ increases according to Equation 3.8 in Chapter 3.
The effect of increasing dispersivity on the total mass of biofilm formed is shown in Figure 6.4. From the graph, it can be seen that with an increasing dispersivity, more biofilm forms during, and in the break after, the pulse of the electron acceptor. The effect is small relative to the total amount of biofilm present. When the electron acceptor becomes limited, the curves overlap, similar to the previous simulation. This is attributed to a lower rate of depletion of the electron acceptor when there is less dispersion, enabling growth to occur for a longer time period.

![Figure 6.4: Biofilm mass accumulation during pulsed injection (with an artificially low decay rate) in a situation where the mechanical dispersion coefficient $\alpha$ is constant, and in one where $\alpha$ increases as a function of the volume fraction of biofilm.](image)

When dispersion increases, injected components remain in the area containing biofilm for a longer time. This is shown in the left column of Figure 6.5. The concentration of substrate in the area next to the injection boundary is slightly higher for the simulation where $\alpha$ increases (the lower image) than where $\alpha$ is constant (top image). The white box for the simulation with a constant value of $\alpha$, highlights the substrate from the previous pulse that was not consumed by the biofilm. When $\alpha$ increases, there is increased spreading, and increased consumption of substrate by the biofilm. As a result of this, the bottom left hand image of Figure 6.5 does not show this pulse of substrate.

When there is more mixing in the zone containing biofilm, more biofilm can accumulate as is shown in the right hand column of Figure 6.5. The volume fraction of biofilm, $\phi_f/\varepsilon$, is slightly higher in the first meter from the injection boundary when $\alpha$ increases (the lower image). Further into the domain, the biofilm distribution appears the same. The effect of increasing dispersivity is most significant near the injection boundary. Using a finer mesh in this area may illustrate the effect of the increasing dispersion.
coefficient more clearly.

### 6.1.3 Decay Rate

Endogenous decay can be modelled using a constant decay rate coefficient, $b_0$, or by a decay rate coefficient that is dependent on the concentration of the electron acceptor, as discussed in Chapter 4, given by Equation 6.1.

$$b_0 = b_e \frac{C_{a,K}}{K_e + C_{a,K}}$$

The purpose of this simulation is to demonstrate the effect of the two options on the total mass of biofilm that accumulates during pulsed injection.

Simulations are carried out, which are identical to the reference scenario, except for the value of $b_0$. The reference scenario uses a constant value of $b_0$, as given in Table 6.1. For the electron acceptor dependent decay rate, the value of $b_e$ is $1.74 \times 10^{-6}$ s$^{-1}$ from Semprini and McCarty (1991), $K_e$ is given in Table 6.1. The mass of the electron acceptor required for endogenous decay of one kg of microbial cells, $d_c$, is 1.42 kg$e$/kg$b$, and the fraction of cells that is biodegradable, $f_d$, is 0.8 after Semprini and McCarty (1991). A third situation is performed, where $b_0$ is also constant, but has a value of $1.74 \times 10^{-6}$ s$^{-1}$, to illustrate the significance of this parameter on the total biofilm accumulation.

The total mass of biofilm in the domain resulting from the three simulations, after 3 injection cycles, is shown in Figure 6.6.

When the decay rate coefficient depends on the availability of the electron acceptor, decay is limited to the periods where the electron acceptor is present and substrate is scarce. This allows for a greater accumulation of biofilm than is possible when the decay rate coefficient is a constant with the value of the maximum decay rate $b_e$ (refer to the curves constant $b_0$ Semprini and $b_0$ dependent on electron acceptor in Figure 6.6). The value for $b_0$ fitted by Ebigbo (2009) is a factor 5.5 less than that by Semprini and McCarty (1991), and allows for biomass to build up to a higher steady state level (this steady state level is approached after a greater number of injection cycles as shown in Figure 6.2). With the high constant decay rate coefficient, almost all accumulated biomass decays between injection pulses. The total mass of biofilm that can accumulate appears quite sensitive to the value of the decay rate parameter. When there is no growth and no endogenous decay, the mass of biofilm in the graph for the electron acceptor dependent decay rate appears constant. Attachment and detachment seem to have little effect on the total mass of biofilm in the system. For the spreading of the biofilm, detachment and attachment can be significant.
6.1 Parameters Affecting Biofilm Development

(a) Concentration of substrate with constant dispersion coefficient $\alpha$. The white box highlights the substrate pulse from the previous injection.

(b) Biofilm distribution, $\phi_f/\varepsilon$, with a constant dispersion coefficient $\alpha$.

(c) Concentration of substrate with increasing dispersion coefficient $\alpha$.

(d) Biofilm distribution, $\phi_f/\varepsilon$, with an increasing dispersion coefficient $\alpha$.

Figure 6.5: Substrate and biofilm distribution for a situation where dispersion coefficient $\alpha$ is constant (top), and one where $\alpha$ increases as a function of the mass fraction of biofilm (bottom). The left column shows substrate concentration at the end of the substrate injection pulse at time = 65 days. The right column shows biofilm distribution when the maximum mass of biofilm is present in the domain at time = 80 days.
Figure 6.6: Biofilm mass accumulation for a decay rate coefficient that is dependent on the availability of electron acceptor, and for constant decay rate coefficients with values fitted by Semprini and McCarty (1991) and Ebigbo (2009).

Whether to model decay dependent on the presence of the electron acceptor depends on the situation that is considered. If an electron acceptor can be assumed to be present at all times, accounting for the consumption of this by endogenous decay may be appropriate. This gives a more complete mass balance for the electron acceptor. In cases where the electron acceptor is limited, constraining endogenous decay to situations where the electron acceptor is present may lead to an over-estimation of the amount of active biomass. For these situations, it appears to be more appropriate to model decay independent of the availability of the electron acceptor.

6.1.4 Injection Strategies

The location of a biofilm barrier and the amount of biomass present when a quasi-steady state is reached, can be manipulated by changing the length of injection pulses, the length of the break between them, and the concentrations that are injected. The qualitative effects of these strategies are demonstrated by simulations in this section.

6.1.4.1 Simultaneous Injection of Substrate and Electron Acceptor

The mass balance for the electron acceptor allows for separate injection of an electron acceptor and a substrate, enabling a range of injection strategies to manipulate biofilm growth. When only substrate is accounted for, the principal variation in injection strategy is a continuous injection versus a pulsed injection. The aim of this simulation is to show the biofilm distribution that can be obtained when only the substrate is accounted for and compare this to the reference simulation.
The parameters and domain are identical to the reference simulation. Two simulations are carried out where the electron acceptor and the substrate are injected simultaneously, representing a model where the electron acceptor is not accounted for. The growth rate of the biofilm is still dependent on the concentration of the electron acceptor, otherwise the growth rate and maximum accumulation of biomass possible would be higher in these simulations than in the reference simulation. The amount of the electron acceptor that is injected, the concentration and the flow rates are the same as for the reference simulation. In one simulation, the electron acceptor and the substrate are injected without a break for 20 days. In the other, 5 day pulses of the substrate and the electron acceptor are injected, followed by 5 day breaks in which only water is injected, and this is repeated for 4 cycles.

The results of the simultaneous injections are shown in Figure 6.7. Biofilm starts to grow near the injection boundary, and all of the substrate and the electron acceptor is consumed in this area. There is no substrate or electron acceptor present at a greater distance from the injection boundary. Suspended and detached microbial cells may attach further downstream, however, they cannot grow and thus biofilm accumulation remains near the injection boundary. Using pulses of injection separated by breaks does not significantly increase the area in which biofilm forms.

![Figure 6.7: Biofilm volume fraction, $\phi_f/\varepsilon$, resulting from simultaneous injection of the electron acceptor and the substrate after 20 days of injection.](image)

(a) Continuous injection of the substrate and the electron acceptor. time = 20 days
(b) Injection of the substrate and the electron acceptor together in pulses. time = 40 days

The distribution of biofilm in the reference simulation, after the substrate and the electron acceptor have been injected for 20 days each, is shown in Figure 6.8. The area affected by biofilm formation is much larger than the area affected when the electron acceptor is injected simultaneously with the substrate. Injected components
are transported by advection before they mix and can be consumed; this results in a greater area where biofilm growth is possible. The highest volume fraction occupied by the biofilm for separate injection is much lower than for simultaneous injection.

Figure 6.8: Biofilm volume fraction, $\phi_f/\varepsilon$, resulting from injection of the electron acceptor and the substrate in separate pulses, after 20 days of injection of each component. time = 76 days

The mass of biofilm present in the domain for the three simulations is shown in Figure 6.9. Comparing the curves showing the mass of biofilm for simultaneous injection, the difference in the maxima is due to decay reducing the mass of biofilm during injection breaks for the pulsed injection. If the amount of biomass in the domain is considered after the same number of days of injection and breaks, the total mass of biofilm present is greater for pulsed injections. The decay rate depends on the amount of biofilm present, thus in the period at the end of the continuous injection, the decay rate is higher than in breaks between the injection pulses. This shows that the two different injection strategies can be used to exert some control on the length of time for which biofilm is present and the volume fraction of the biofilm present in the domain.

Pulsed injection results in significantly greater spreading of the biofilm through the domain. This is at the cost of a greater fraction of injected solutes not being consumed. With the same mass and concentration of solutes injected, the mass of biofilm formed during simultaneous injection is higher. However, the only control that can be exerted by injection strategies during simultaneous injection, affects the length of time for which biofilm is present, not the spatial distribution of the biofilm.
6.1 Parameters Affecting Biofilm Development

6.1.4.2 Injection of Water Between Pulses

When water is injected between injection pulses, components travel further from the injection boundary before mixing. The purpose of this simulation is to investigate the effect of injecting water during injection breaks on the distribution of biofilm in the domain.

Two simulations are performed, with the same parameters and initial conditions as the reference simulation. The pulse length is 5 days, and the breaks are 10 days, 5 complete cycles are injected. The flow rate of water and concentrations of the components are the same as for the reference situation. Biomass is injected in the first 5 days of the first pulse of the electron acceptor. For one of the simulations, water is injected continuously, which is also done in the reference simulation, for the other, the injection of water is stopped in the breaks when no solutes are injected.

Without injection of water, the substrate and the electron acceptor mix near the injection boundary and biofilm forms thickest in this zone, as shown in Figure 6.11. The area affected by biofilm is increased by continuous injection of water, however volume fraction of biofilm, and thus the permeability reduction, near the injection well is less. The effect of injecting water in the breaks between pulses on the distribution of biofilm appears to be quite significant.

Figure 6.9: The total mass of biofilm resulting from simultaneous continuous injection, simultaneous pulsed injection, and separate pulsed injection, for the same total mass of the electron acceptor and the substrate.
6.1 Parameters Affecting Biofilm Development

6.1.4.3 Length of Injection Breaks

Varying the length of the break between pulses of the substrate and the electron acceptor changes the distance that the injected components travel before they mix, and thus affects the area where biofilm can form. The aim of these simulations is to qualitatively evaluate the effect of changing the break length.

The simulations are identical to the reference simulation, only the length of the break is varied. One simulation is performed with no breaks, one with 3 day breaks, and one with 10 day breaks. The simulations last for 5 injection cycles.

Increasing the length of the breaks results in an increase in the area affected by biofilm growth, as shown in Figure 6.11. This indicates that the area in which the electron acceptor and the substrate are present simultaneously is increased by longer breaks. The fraction of the pore volume occupied by biofilm in the area where the biofilm accumulation is highest, is lower for longer break lengths. The transition from porous medium containing biofilm to clean porous medium also becomes more gradual when the length of the breaks increases. Biofilm appears to be spread more homogeneously through the domain.

With longer breaks, the mass of biofilm present when the total mass of biofilm in the domain is at a maximum, is reduced, as shown in Figure 6.12. With longer breaks, there is a longer period of decay in the injection cycle, thus the total mass that accumulates in each cycle is less. Additionally, more of the electron acceptor and the substrate flow out of the domain. When a given injection strategy is applied over a sufficiently long
Figure 6.11: Biofilm volume fraction, $\phi_f/\varepsilon$, as a result of different break lengths for a constant pulse length.
time, the quasi-steady state accumulation of biofilm is highest when the breaks are shortest.

![Image](image.png)

Figure 6.12: Effect of changing the length of the break between the injection pulses on the total mass of biofilm present in the system.

### 6.1.4.4 Pulse Length

The length of the pulses can also be varied to manipulate the biofilm distribution. The purpose of these simulations is to investigate the effect of varying the pulse length, and consider the relation between pulse and break lengths.

The simulations that are carried out are identical to the reference simulation, except for the lengths of the injection pulses and breaks. The injection strategies that are carried out are: 3 day injections with 5 day breaks; 10 day injections with 5 day breaks; 5 day injections with 10 day breaks; and 10 day injections with 10 day breaks. The electron acceptor and the substrate are injected for 25 days in total for each of the simulations.

The total area where biofilm forms is greater when the pulse length is longer for simulations with a 5 day break, as shown by the upper two figures in Figure 6.13. The transition between the area where biofilm is present and clean porous medium is more gradual than for longer injection periods (for the distribution resulting from 5 day pulses with a 5 day break please refer to Figure 6.8, the reference simulation).

When the breaks are 10 days, increasing the length of the pulses results in a higher accumulation near the injection point, but not in a significantly greater area affected by biofilm, as shown in the lower two images of Figure 6.13. The zone where the volume fraction of biofilm is highest, is not next to the injection point but some distance from it when the pulse length is 5 days and the break is 10 days.
Considering the effect of 10 day pulses, for 5 and for 10 day breaks (the right column of Figure 6.13) it must be noted that the total mass of biofilm is greater with 5 day breaks. The scales for these figures have been selected to enable comparison between the figures with equal break lengths.

In Figure 6.14 the total mass of biofilm in the system is shown for injection lengths of 3, 5 and 10 days, with a 5 day break between pulses. The same amount of the electron acceptor and the substrate are injected in each simulation. Increasing the pulse length for these simulations results in a greater mass of biofilm formed. Increasing the pulse length beyond 10 days leads to a decrease in the total mass of biofilm formed. For this size of the domain the boundary conditions on the eastern boundary influence the solution.

\begin{figure}
\centering
\begin{minipage}{0.45\textwidth}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{3 day pulses with 5 day breaks. time $t = 141$ days}
\end{minipage}\hfill
\begin{minipage}{0.45\textwidth}
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{10 day pulses with 5 day breaks. time $t = 82$ days}
\end{minipage}
\begin{minipage}{0.45\textwidth}
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{5 day pulses with 10 day breaks. time $t = 109$ days}
\end{minipage}\hfill
\begin{minipage}{0.45\textwidth}
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{10 day pulses with 10 day breaks. time $t = 143$ days}
\end{minipage}
\caption{Biofilm volume fraction, $\phi_f/\varepsilon$, as a result of different pulse lengths.}
\end{figure}

The difference in the total mass of biofilm appears to be more strongly affected by changing the break length than by changing the pulse length, as can be seen by com-
Figure 6.14: Effect of varying the pulse length on the total mass of biofilm present in the system.

Comparing Figures 6.12 and 6.14. When the pulse length is increased, the length of time during which growth takes place increases relative to the length of the decay phase, resulting in more biofilm accumulation in each injection cycle. However, increasing the pulse length also results in a greater fraction of the solutes not mixing at all, which leads to less biofilm accumulation.

When the break length is increased, this results in both a longer decay period relative to the growth period, and a greater fraction of the injected components does not mix at all. This can explain why the effect of increasing the break length is more pronounced than the effect of changing the pulse length.

6.1.4.5 Concentration of Electron Acceptor and Substrate

The growth rate coefficient of biomass is related to the concentrations of the electron acceptor and the substrate by double Monod kinetics (refer to Chapter 4). The aim of this simulation is to assess the effect of increasing the injected concentration of both solutes on the total mass and distribution of biofilm formed.

The simulation is identical to the reference simulation, except that the concentrations of the electron acceptor and the substrate are doubled.

In Figure 6.15 it can be seen that doubling the concentrations results in more than doubling of the total mass of biofilm formed at the quasi-steady state. This can be attributed to an increased mixing of the components. When higher concentrations of solute are injected, these disperse faster, as hydrodynamic dispersion is dependent on the concentration gradient. More mixing results in higher concentrations of the electron
acceptor and the substrate in the same area, and a higher biofilm growth rate. The length of time that biofilm grows during each injection cycle is the same in the reference simulation and when the concentrations are doubled. The higher accumulation is due to only a higher growth rate, and not a longer period where the two components are present in these simulations.

The area that is affected by biofilm is not twice as large when the injected concentrations are doubled, but a higher volume fraction accumulates, as shown in Figure 6.16. The difference between the volume fraction of biofilm in the two simulations appears greatest near the injection boundary and decrease with distance into the domain.

![Figure 6.15: Effect of doubling the injected concentration of the electron acceptor and the substrate on the total mass of biofilm.](image)

In the preceding simulations, the electron acceptor is the limiting reagent, as it is consumed at a higher rate by the biofilm and equal concentrations of the electron acceptor and the substrate are injected. When the concentration of the electron acceptor is twice the concentration of the substrate, the components are injected at nearly the stoichiometric ratio required for biomass growth. The purpose of this simulation is to see how the accumulation of biofilm changes when only the concentration of the electron acceptor is varied.

The simulations are identical to the reference simulation, only the concentration of substrate is 0.25 kg/m$^3$, and the electron acceptor is injected with concentrations of 0.25 kg/m$^3$, 0.5 kg/m$^3$, and 1.0 kg/m$^3$. 3 injection cycles are simulated. Increasing the concentration of the electron acceptor increases the growth rate and results in a greater total mass of biofilm, as shown in Figure 6.17. With a higher concentration of the electron acceptor, growth continues for a longer time and at a higher
6.1 Parameters Affecting Biofilm Development

6.1.5 Lysis due to Dissolved CO₂

The potential of dissolved CO₂ to cause lysis of microbial cells is discussed in Chapter 5. The purpose of these simulations is to investigate the consequences of this for the accumulation and distribution of biofilm.

Simulations are performed where the biofilm volume fraction at the start of the simulation is 0.05 in the entire domain. The other initial conditions are as for the reference simulation. CO₂ is injected along the entire west boundary at a constant rate of 0.0001 kg/(m²s). The boundary conditions for the remaining three boundaries are as in the reference simulation. One simulation is carried out where both lysis coefficients, \( c_{c1} \) and \( c_{c2} \), are zero, and only endogenous decay takes place. In the remaining simulations, \( c_{c1} \) has the value fitted by Ebigbo (2009), shown in Table 6.1. The decay rate parameter, \( c_{c2} \), accounting for lysis due to dissolved CO₂ takes values of: 0, 0.05, 0.1, 0.2, 0.5 and 1.0 days⁻¹.

In Chapter 4 it is shown that CO₂ has a greater effect on suspended cells than on cells in a biofilm. To account for this a a lower threshold mass fraction, \( X_{tox,r} \), is used for suspended cells. The values used are 0.03 kg/kg for suspended cells, and 0.04 kg/kg for the biofilm. The concentration of dissolved CO₂ is equal to the maximum solubility of CO₂ in brine for the greatest part of the area where dissolved CO₂ is present, as shown the right image of Figure 6.20. Variations to the values of \( X_{tox,r} \) will have little
6.1 Parameters Affecting Biofilm Development

Figure 6.17: Effect of varying the concentration of the electron acceptor injected, for a constant injection concentration of substrate 0.25 kg/m³, on the mass of biofilm present.

effect on the simulation results. The remaining parameters are the same as for the reference simulation.

The results of the simulations are plotted in Figure 6.18. The effect of lysis due to gaseous CO₂ alone is very small compared to the endogenous decay rate. The curve with no lysis, overlaps the curve where lysis is only due to gaseous CO₂ ($c_2$ is 0.0 days⁻¹). The simulation where $c_2 = 1.0$ days⁻¹ results in a curve touching the curve for 0.5 days⁻¹.

For higher values of $c_2$, the time taken for all biofilm to decay is shorter, the area that is affected is larger, and the interface of the lysed zone is sharper, as shown in Figure 6.19. Including lysis due to dissolved CO₂, causes a more significant reduction in biofilm volume and reduces the volume over a larger area than when lysis is only due to gaseous CO₂.

In order for supercritical CO₂ to cause lysis of cells in the biofilm, free-phase CO₂ must be present in the pores of the biofilm. The entry pressure of Continuum $F$ is higher than of Continuum $P$, and the saturation of the CO₂ phase must be relatively high in Continuum $P$, before it enters Continuum $F$ (refer to Figure 1.6 in Chapter II). The saturation of free-phase CO₂ for these simulations is shown in the left image of Figure 6.20. The maximum saturation is below 0.2, at this saturation the amount of free-phase CO₂ in Continuum $F$ is neglectable. Therefore, little lysis due to supercritical CO₂ occurs in the biofilm. The mass fraction of dissolved CO₂ in Continuum $F$, is shown in the right image of Figure 6.20. This is above the level at
Figure 6.18: The total mass of biofilm present during continuous CO$_2$ injection, with different values for the decay coefficient, $c_{c2}$, and a constant value of $c_{c1}$, as well as the mass of biofilm in a situation without lysis and only endogenous decay.

which it is assumed to significantly affect bacterial cells, i.e. 0.04 kg/m$^3$, in the first 3 meters of the domain. Thus, if dissolved CO$_2$ causes lysis this can have a significant effect as it penetrates the biofilm much faster than free-phase CO$_2$.

### 6.1.6 Co-Injection of CO$_2$ and Electron Acceptor

The electron acceptor can be injected as a component of the water phase as well as as a component of the CO$_2$ phase. If the substrate is present in the aquifer, co-injecting the electron acceptor with CO$_2$ may stimulate biofilm growth. The injected electron acceptor dissolves in water and can be consumed by cells in the biofilm. The aim of this simulation is to investigate what effect this can have on the accumulation of biomass in the system.

For this simulation, the same conditions are used as for the simulations in Section 6.1.5 except that the initial concentration of substrate is 0.005 kg/m$^3$ in the entire domain. The value of $c_{c1}$ is given in Table 6.1. $c_{c2}$ is 0.05 days$^{-1}$. CO$_2$, containing 0.7 kg/m$^3$ of the electron acceptor, oxygen, is injected at a rate of 0.0001 kg/(m$^2$s).

The effect of injecting the electron acceptor on the total mass of biofilm present is very small. There is no distinction between the curve plotting biofilm mass when the electron acceptor was injected and the curve when it was not. Endogenous decay and lysis significantly reduce the total amount of biofilm in the system, as shown by Figure 6.18, this outweighs the effect of additional growth due to the injected electron acceptor.
Figure 6.19: Distribution of biofilm, $\phi_f/\varepsilon$, after 15 days of CO$_2$ injection for different values of $c_{c2}$.

Figure 6.20: The saturation of the CO$_2$ phase and the mass fraction of dissolved CO$_2$ in the water phase in Continuum $F$ are shown after 15 days of CO$_2$ injection.
The effect on the biofilm distribution can be seen in Figure 6.21, this is also minimal. There is a zone near the injection boundary where biofilm growth compensates to some extent for decay and lysis in the left image. Dissolved CO\(_2\) diffuses in Continuum \(F\) at a similar rate as the electron acceptor, and causes cell lysis, leaving less active bacteria to consume the electron acceptor. As injection proceeds for a longer time, the difference in the biofilm distribution between the two scenarios diminishes.

![Figure 6.21: Biofilm distribution, \(\phi_f/\varepsilon\), during CO\(_2\) injection with and without the electron acceptor being co-injected after 3 days of injection.](image)

6.2 Parameters Affecting Two-Phase Flow

The change in pore geometry of a porous medium due to biofilm formation, is related to the parameters that describe two-phase flow in Chapter 3. Relations are proposed for the changes in pore size distribution index, \(\lambda_p\), and the entry pressure, \(p_{d,p}\), for Continuum \(P\). Equations 1.9 and 1.10 describing two-phase flow (refer to Section 1.3), can be rewritten to express relative permeability in terms of \(p_e\), \(p_d\) and \(\lambda_p\), as shown in Equation (6.2).

\[
\begin{align*}
    k_{rw} &= \frac{p_d}{p_e} \left( \frac{2 + 3\lambda}{2 + \lambda} \right) \\
    k_{rn} &= \left( 1 - \frac{p_d}{p_e} \right)^2 \left( 1 - \frac{p_d}{p_e} \right)^{2 + \lambda} \quad (6.2)
\end{align*}
\]

In this section, the effect of the changes made in Chapter 3 on the model outcomes is assessed qualitatively.

6.2.1 Pore Size Distribution Index

The value of \(\lambda_p\) rises fastest when biofilm starts to accumulate (refer to Figure 3.4 in Chapter 3). When the effective saturation is the same, an increase in \(\lambda_p\) causes a
reduction in the capillary pressure of Continuum $P$ (refer to Figure 3.3 in Chapter 3), and an increase in the relative permeability of the non-wetting phase in Continuum $P$, as shown by Equation 6.2. The purpose of this simulation is to investigate to what extent the model predictions are affected by the relation for $\lambda_p$. In the area where biofilm grows, for a constant effective saturation, the capillary pressure of Continuum $P$ decreases in the simulation where $\lambda_p$ increases. A lower capillary pressure in Continuum $P$, implies more of the non-wetting phase must be present in Continuum $P$ before it enters Continuum $F$. The non-wetting phase can only enter Continuum $F$ when the capillary pressure in Continuum $P$ is equal to, or greater than, the entry pressure of Continuum $F$. When less free-phase CO$_2$ enters Continuum $F$, less lysis of the biofilm is expected. However, an increase in the relative permeability of the non-wetting phase in the area containing biofilm, may result in a greater flux of the gas phase into this zone.

### 6.2.1.1 Model Domain and Parameters

In this simulation, a biofilm is grown in the top of the aquifer by pulsed injection; after a period of time CO$_2$ is injected below this. As biofilm degrades quickly due to lack of substrate, pulsed injection is continued during the CO$_2$ injection. The model domain and the injection strategy are the same as for the reference simulation in Section 6.1.1 except that the injection rate of water is 0.0005 kg/(m$^2$s), and injection is only in the upper 5 metres of the domain. CO$_2$ injection starts after 100 days in the lower 5 metres of the domain at a rate of 0.00005 kg/(m$^2$s). The boundary and initial conditions are the same as in Section 6.1.1. Gravity is included to account for flow driven by density differences of the fluids.

The model parameters are the same as in Section 6.1.1 except for the hydraulic properties of the porous medium. The changes to $\lambda_p$ and $p_d$ are proposed based on the properties of sandstone aquifers, the parameters used for this are given in Table 6.7.

<table>
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<td>Permeability</td>
<td>$5.0 \times 10^{-13}$ m$^2$</td>
</tr>
<tr>
<td>Porosity</td>
<td>0.22</td>
</tr>
<tr>
<td>Specific surface of aquifer $M$</td>
<td>$2.2 \times 10^4$ m$^{-1}$</td>
</tr>
<tr>
<td>Permeability parameter $\phi_{p,c}/\phi_0$</td>
<td>0.6</td>
</tr>
<tr>
<td>Permeability parameter $n_k$</td>
<td>3</td>
</tr>
<tr>
<td>Minimum permeability $K_{min}$</td>
<td>$0.01 \cdot K_0$</td>
</tr>
</tbody>
</table>

A simulation is performed where $\lambda_p$ increases and one where $\lambda_p$ is constant. Biofilm accumulation reduces the intrinsic permeability to the same extent in both simulations. In the simulations in Section 6.1.5 pulsed injection is stopped and CO$_2$ is injected
directly into the biofilm. The high decay rates resulting from both endogenous decay and lysis cause a fast reduction in the mass of biofilm. In this simulation, continued injection of the substrate and the electron acceptor compensates for endogenous decay. The continued injection of water also reduces the concentration of dissolved CO$_2$ in the biofilm. Therefore the rate of lysis due to dissolved CO$_2$ is reduced. Lysis due to gaseous CO$_2$ is also reduced, as CO$_2$ flow into the biofilm is only driven by a density difference between the fluids. These conditions are chosen in order to maintain biofilm in the aquifer during CO$_2$ injection, to demonstrate the effect of an increase of $\lambda_p$ caused by the presence of biofilm.

The simulation results do not show a clear difference between the saturation of the CO$_2$ phase in the two situations. Water injection during this simulation prevents the flow of CO$_2$ into the biofilm to some extent. If CO$_2$ could rise into the biofilm affected zone easier, this might give a greater difference between the saturation of the CO$_2$ phase in the two simulations. Without injection of nutrients, the biofilm decays at such a rate that the effect on $\lambda_p$ is diminished before a change in gas saturation can become observable.

The effect of increasing $\lambda_p$ on the accumulation of biofilm is shown in Figure 6.22. During CO$_2$ injection, a slightly greater mass of biofilm is present in the case where $\lambda_p$ increases. This is in agreement with the expectation that less supercritical CO$_2$ enters Continuum F due to capillary forces, reducing the amount of lysis. The difference between the total mass of biofilm in the two simulations is very small.

Figure 6.22: Total mass of biofilm, with CO$_2$ injection starting after 100 days, for a situation where $\lambda_p$ is a constant and one where $\lambda_p$ increases with $\phi_f/\varepsilon$. 

\[
\text{total mass biofilm} \quad [\text{kg}] \\
\]
6.2.2 Entry Pressure

When the entry pressure of Continuum $P$ is increased by the formation biofilm, this reduces the relative permeability of the CO$_2$ phase as given by Equation 6.2. Simulations with the same conditions that were used to demonstrate the effect of changes in $\lambda_p$, were performed for a value of $p_d$ that was constant and one that increases according to Equation 3.7 (refer to Chapter 3).

The model output does not show a difference in the saturation of the CO$_2$ phase or in biofilm mass. The effect of biofilm formation on entry pressure becomes significant when a relatively large fraction of the pore space is occupied by biofilm. In these simulations, the greatest fraction of the pore volume occupied by biofilm is 0.01. This results in an increase in $p_d$ from 0.1 bar to 0.1005 bar. This reduces the relative permeability of the CO$_2$ phase by a factor 1.03, which is too little to be observed at this mesh size. Noticeable changes in $p_d$ occur only when the intrinsic permeability of Continuum $P$ has been reduced to such an extent that simulations, with this mesh size, do not show any effects.

6.3 Summary

The simulations performed in this chapter are intended to investigate the effects of various modifications made to the model, and the effect of some injection strategies. No mesh convergence study has been done, this would be an appropriate next step in order to be able to assess the importance of various parameters.

Simulations indicate that for a constant injection strategy, biofilm accumulation results in a quasi-steady state mass of biofilm in the aquifer. This is also found using other models that consider pulsed injection of the electron acceptor and the substrate (Rockhold et al., 2005; Semprini and McCarty, 1991).

The addition of dispersive mixing to the model has a noticeable effect on the amount of biofilm formed. Increases in the dispersivity parameter $\alpha$ resulting from biofilm accumulation show less pronounced effects. The effect of numerical dispersion on the outcomes has not been investigated. Performing simulations with a smaller mesh size could reduce the degree of numerical dispersion, so that the effect of mechanical dispersion becomes more pronounced.

The endogenous decay rate parameter has a large effect on the mass of biofilm present at a quasi-steady state. It is suggested that for CO$_2$ storage, it is appropriate to have a constant endogenous decay rate parameter, to avoid an overprediction of the amount of active biomass in the system. When the electron acceptor is more abundant, and the mass balance of the electron acceptor is of key interest, modelling the consumption
of the electron acceptor for endogenous decay may become desirable.

The effect of different injection strategies is investigated, and compared qualitatively to a reference simulation. Simultaneous injection of the electron acceptor and the substrate results in accumulation of biofilm near the injection point. Different injection strategies, continuous and pulsed injection, give little control over the distribution of biofilm in that situation. The distribution can be influenced when the electron acceptor and the substrate are injected in separate pulses, particularly when water is also injected in the breaks between pulses. Increasing the length of the breaks leads to a greater spreading of biofilm in the domain. In this case, there is a trade off, between the size of the area where biofilm grows, and the total mass of biofilm that is formed. For longer pulses, a greater total mass of biofilm accumulates, and the area affected is larger. Increasing the pulse length has a less pronounced effect on the distribution and total mass of biofilm formed than increasing the break length does. When the length of either the break or the pulse, is increased, a greater portion of the injected components does not mix. When the pulse length is increased, however, this results in a longer period of time in which biofilm growth takes place, relative to the time in which decay takes place. This partially offsets the effects of the reduction in growth. For simulations with long pulses and a long breaks, the size of the domain may have affected the results. When the injection pulse and the break both last 10 days, nearly the entire domain contains biofilm and the eastern boundary conditions may influence the model outcomes.

Doubling the concentrations of both the substrate and the electron acceptor more than doubles the mass of biofilm that forms. With higher concentrations, there is more diffusive mixing of the components, and the growth rate of biomass increases. This results in a higher consumption of the electron acceptor and the substrate by the biofilm. The maximum concentration of the components that can be injected is determined by their solubility.

Increasing the concentration of the electron acceptor relative to the concentration of the substrate, shows that with excess electron acceptor more biofilm forms than when the two are injected at the stoichiometric ratio for biofilm growth. This can be attributed to greater dispersion of the electron acceptor causing a higher biofilm growth rate.

When lysis by dissolved CO$_2$ is accounted for, this has a significant effect on the total mass and distribution of biofilm. Due to capillary forces, the supercritical CO$_2$ only enters the biofilm when the saturation of the CO$_2$ phase in the porous medium is high. Particularly at lower injection rates, the front of dissolved CO$_2$ travels ahead of the CO$_2$ phase, and causes lysis to a greater extent than the supercritical CO$_2$ does. The co-injection of the electron acceptor with CO$_2$ has little effect on the total amount of
biofilm present. Lysis and endogenous decay cause a fast reduction in the total mass of biofilm, and the additional growth is negligible.

The effect of changing the hydraulic parameters of the porous medium as a function of biofilm accumulation appears to be relatively small. At the mesh size used, changes to neither $\lambda_p$ nor $p_d$ appear to have a significant effect on the saturation of the CO$_2$ phase. $\lambda_p$ changes faster when a lower mass of biofilm is present, and the effect of this on the mass of biofilm present can be seen in the model outcomes. $p_d$ changes only when the intrinsic permeability has been reduced to such an extent that its effects on two-phase flow and on biofilm accumulation appear to be negligible.
Chapter 7
Evaluation

In this work, an existing model is adapted in order to increase its potential to simulate the growth of engineered biofilms. In addition to this, some adaptations are made to the hydraulic parameters that reflect the pore scale effects of biofilm growth. As the model is intended primarily for CO$_2$ injection, potential damage to the biofilm by dissolved CO$_2$ is also considered.

The principal aim of this work is to increase the applicability of the model by extending it to enable separate injection of the electron acceptor. The resulting model is used to simulate the effect of different injection strategies. These all show that separate injection leads to a more homogenous distribution of biofilm, and less clogging near the injection boundary, than when the substrate and electron acceptor are injected together. The outcomes of various simulations indicate that using a constant injection strategy, for a sufficiently long time, results in a quasi-steady state mass of biofilm in the domain. This corresponds to field observations reported by Semprini and McCarty (1991). The amount of biofilm present at the steady state as well as its spatial distribution can be manipulated by changing the concentration of solutes, the length of injection pulses, and the length of the breaks between the pulses. Based on this, strategies can be selected that make the most efficient use of the substrate and the electron acceptor to form the desired biofilm barrier.

An important assumption made here is that only one type of electron acceptor is consumed by the biomass. If multiple electron acceptors are present naturally, this can result in biofilm growth when only substrate is injected. This may reduce the extent to which the biofilm distribution can be controlled by pulsed injection. The effect of this depends on the amount of the electron acceptors present and the rate at which they are used up.

Oxygen is chosen as the electron acceptor to inject, as this gives a high energy yield and is used preferentially by many microorganisms. Investigations by McLean et al. (2008) indicate that biofilms of Shewanella oneidensis grown in air can switch from oxygen
to other electron acceptors, without a lag in growth, when oxygen becomes limited. Parks (2009) found that suspended cells of *S. pasteuri* remained active under anaerobic conditions, however, it could not be demonstrated that the cells were growing.

To account for potential growth due to other electron acceptors when oxygen is limited, a growth rate that is only dependent on the concentration of substrate can be implemented. When oxygen is present, the bacteria grow at a rate modelled by double Monod kinetics as implemented in Chapter 4. If the concentration of oxygen drops below a minimum level, microorganisms can be assumed to switch to a different electron acceptor, and continue growing at a reduced rate. This assumption would only be valid if it can be shown that the alternative electron acceptor does not get limited after a certain length of time.

Field data is needed to verify the model, and to indicate whether the presence of electron acceptors in the aquifer has a significant effect on biofilm growth. The kinetic parameters used in the model depend to a large extent on the in situ conditions and the types of microorganism that are used. These parameters should be fitted to field data in order to make quantitative predictions. Simulations indicate that kinetic parameters play an important role in determining the mass of biofilm that can accumulate. A sensitivity study, to determine the importance of the various parameters, associated particularly to biofilm growth, is also needed for this model.

The second goal of this work, is to relate biofilm growth to the hydraulic properties of the porous medium, based on pore scale considerations of biofilm accumulation. Controversy exists regarding the growth pattern of biofilms. In this work, several hypotheses regarding biofilm formation are combined in a conceptual model describing biofilm accumulation. This model is used to relate biofilm accumulation to three macroscale hydraulic parameters, dispersivity, pore size distribution index and entry pressure.

Mechanical dispersion itself has a noticeable effect. It increases mixing of the electron acceptor and the substrate and enables slightly more biofilm accumulation. Increases in mechanical dispersivity related to biofilm growth have a minimal effect on solute mixing. Refining the mesh, to reduce the effect of numerical dispersion, may bring out this effect. The changes to the pore size distribution index and the entry pressure appear to have little effect on two-phase flow with the domain and mesh used. A mesh convergence study has not been performed, this could be a next step.

The growth pattern that is assumed for biofilm formation on the pore scale has little effect on the results of this model for two-phase flow. The reduction in intrinsic permeability is modelled without making assumptions for a biofilm growth pattern, and the effect of this is dominant. For the computational efficiency of the model, therefore, it may be preferable to assume constant values of pore size distribution index and entry pressure. The effect of a higher dispersion coefficient in the area affected by biofilm may be worth investigating further, particularly if, in a further extension of the model, biomineralisation is to be considered. Increased dispersion
of solutes in the area containing biofilm should result in a greater mixing of the components used for precipitation by the biofilm.

Literature regarding sterilisation of microorganisms by CO$_2$ is studied to assess whether dissolved CO$_2$ can affect the integrity of the biofilm. It is suggested that, due to the high pressure in the aquifer, the concentration of dissolved CO$_2$ can be high enough to cause damage to microbial cells. The values of the parameters used to model this have to be determined from experimental data. The effect of dissolved CO$_2$, even if the maximum lysis rate is 5 orders of magnitude smaller than the maximum lysis rate of supercritical CO$_2$, can be quite significant, as dissolved CO$_2$ can invade the biofilm faster than free-phase CO$_2$. Therefore, the dissolution of CO$_2$ and its effects on the biofilm are considered important to include in the model.

The purpose of growing a biofilm is to reduce the permeability of the aquifer, so that CO$_2$ can be safely stored. When injection of the substrate and the electron acceptor is stopped, endogenous decay reduces the volume of biofilm and the permeability recovers. When CO$_2$ is injected, lysis causes the biofilm to degrade faster. Co-injecting the electron acceptor with CO$_2$, in an aquifer where substrate is present, does little to compensate this decay. In order to form a more permanent seal, biomineralisation is considered. Not the biofilm itself reduces the permeability, but a mineral, like calcium carbonate, that is precipitated by the biofilm. To model this, it is important to be able to model the distribution of the biofilm.

Further applications of this model could be in the field of groundwater remediation. The pollutant may be the electron acceptor, and injections of biomass, substrate, and water can be used to stimulate the growth of a bio-barrier. In this case, it may be desirable to model an endogenous decay rate that is dependent on the concentration of the electron acceptor. This allows a more accurate treatment of the mass the electron acceptor. Both the containment of a pollution plume, due to reduced permeability of the formation, and the degradation of the pollutant due to consumption by the biofilm can be simulated by this model.


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<td>Cao, Y. / Eikemo, B. / Helmig, R.</td>
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<td>Simulating the effect of capillary flux on the soil water balance in a stochastic ecohydrological framework</td>
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