Supporting Information

Experimental results and simulations of bacterial growth on insoluble hydrophobic substrate (phenanthrene)

Adam I.K.U., Rein A., Miltner A., Fulgencio A.C.D., Trapp S., Kästner M.

Content

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- - Data on the statistical evaluation of the model curve fits (Table S2)

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Including the figures:

Figures SI 1, 2, 3, 4. Kinetic data for the strains *Sphingomonas* sp. EPA505 and *Sphingobium yanoikuyae*

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Surface area of crystals

The surface area A_{ph} is obtained from PHE mass and a transient correction factor for the change in size of the crystals f_A (-),

$$A_{ph} = 6 \times V_{ph}^{2/3} \times f_A$$

with

$$V_{ph} = \frac{m_{ph}}{\rho_{ph} \times 1000}$$

and

$$f_A = f_{A,0} \times e^{-k_{agg} \times t}$$

where V_{ph} (m^3) is the volume of the organic (PHE) phase and ρ_{ph} (kg m^{-3}) is phase density. The correction factor f_A represents the difference between (generically assumed) cubic geometry and the effective surface area, as well as possible exponential decrease of exchange surface with time due to the coagulation and dissolution of microcrystals, with initial ratio $f_{A,0}$ (-) and first-order rate constant for aggregation k_{agg} (d^{-1}).

Consideration of initial lag phases in the mathematical model

Initial lag phases are often observed during degradation experiments, i.e. degradation is not starting immediately but gradually, which can be explained by an adjustment of microbes to new conditions. Such effects need to be considered in the model approach in order to explain early observations in degradation experiments. This can be done using a transient (temporally decreasing) inhibition factor f_{inh}, leading to a gradual increase of biodegradation until full

degradation capacity is reached (which would correspond to f_{inh} = 1).[1] Similarly[2, 3] Eq. (2) is modified accordingly to:

$$\frac{dm_M}{dt} = \frac{v_{\max} \times C_W}{K_M + C_W} \times X \times f_{inh}$$

In this work, the microbes have been grown in pre-cultures (on phenanthrene) prior to their use in the degradation experiments and it is likely that during that phase, phenanthrene has been incorporated within the cells. After transfer into the experimental vessels, this amount of incorporated phenanthrene is degraded first, before phenanthrene in suspension is used by the microbes, which causes an inhibition of substrate uptake. This is compensated for by the inhibition factor f_{inh} (-):

$$f_{inh} = \frac{m_{ph,cell}(0) \times f_{inh,corr} - m_{ph,cell}(t)}{m_{ph,cell}(0) \times f_{inh,corr}}$$

where phenanthrene within cells is $m_{ph,cell}$ (initial mass $m_{ph,cell}$ (0), corresponding to maximum mass) and the correction factor for inhibition is $f_{inh,corr}$. Thus v_{max} is inhibited and this inhibition is decreasing with time until the substrate stored within the cell from the pre-culture is consumed; in other words, it reflects the time needed to consume the phenanthrene introduced into the experiment with the inoculation of the cells. The corresponding amount of “new” phenanthrene (from the degradation experiment) has to flow into the cells; this also may take time. A $f_{inh,corr}$–value of 1 in this equation assumes that the whole pool of phenanthrene mass present in suspension is available for uptake into cells (replacing the pre-culture amount of phenanthrene). Varying the correction factor $f_{inh,corr}$ can account e.g. for lower portions available for the transfer into cells, or for differences in dissolution from the solid phenanthrene phase.

Details of the determination of kinetic parameters

The “true” yield differs from the experimentally derived yield Y_{exp}, which is usually the ratio of observed growth dX to observed change of substrate mass dm_M. If $dm_M\ dt^{-1}$ is determined only by $v \times X$:

$$Y_{\exp} = \frac{dX}{dm_M} = \frac{\mu - b}{v}$$

i.e. maintenance r is missing in the denominator. As the maximum substrate consumption v_{max} is typically more than a factor 10 larger than the resources needed for maintenance r, the “true” yield is often approximated (with an error < 10%) from the maximum observed growth v^{obs}_{max}:

$$Y \approx \frac{dX_{\max} / \left(X\ dt\right)}{v^{obs}_{\max}} = \frac{\mu_{\max} - b}{v^{obs}_{\max}}$$

Thus, prior to determining Y, the death rate constant b was fitted at low substrate concentration. In a next set of experiments with very high substrate concentrations, first μ_{max} was determined from observed $dX_{max}\ (X\ dt)^{-1}$ and known b ($\mu_{max} = dX_{max}\ (X\ dt)^{-1} + b$), and then Y with observed v^{obs}_{max}; v_{max} can be calculated ($v_{max} = \mu_{max} \times Y^{-1}$). Finally, in experiments with moderate substrate concentrations K_M can be fitted.

Table SI 1. Parameters for dissolution from phenanthrene phase fitted for the different experiments.

Experiment (nominal initial phenanthrene concentration C_0)	$f_{A,0}$ **(-)**	k_{agg} **(d^{-1})**	$f_{inh,corr}$ **(-)**
Novosphingobium pentaromativorans			
C_0 = 10 mg L^{-1}	110	0.6	10
C_0 = 25 mg L^{-1}	60	0.4	4
C_0 = 50 mg L^{-1}	80	0.5	10^{-16}
C_0 = 100 mg L^{-1}	70	0.4	0.5
C_0 = 200 mg L^{-1}	70	0.15	0.15
C_0 = 400 mg L^{-1}	160	0.5	0.2
Sphingomonas sp. EPA505			
C_0 = 10 mg L^{-1}	110	0	1
C_0 = 25 mg L^{-1}	100	0	10^{-16}
C_0 = 50 mg L^{-1}	140	0	10^{-16}
C_0 = 100 mg L^{-1}	120	0.5	10^{-16}
C_0 = 200 mg L^{-1}	120	0.5	10^{-16}
C_0 = 400 mg L^{-1}	75	0.3	10^{-16}
Sphingobium yanoikuyae			
C_0 = 10 mg L^{-1}	80	1.7	15
C_0 = 25 mg L^{-1}	200	2.2	10^{-16}
C_0 = 50 mg L^{-1}	80	0	10^{-16}
C_0 = 100 mg L^{-1}	150	0.6	10^{-16}
C_0 = 200 mg L^{-1}	80	0	0.01
C_0 = 400 mg L^{-1}	80	0.3	0.02

$f_{A,0}$: factor to compensate for the difference between (generically assumed) cubic geometry and the"real" phenanthrene surface area, initial value; k_{agg}: first-order co-angulation rate constant, describing the decrease of phenanthrene surface area (decrease of $f_{A,0}$) with time; $f_{inh,corr}$: factor scaling inhibition due to lag phase, 10^{-16} represents a very small number (no inhibition). See main article for details.

Table SI 2. Statistical evaluation of the model curve fits (results shown in Figure 1 and 2, Figure SI 1 to SI 4) for phenanthrene concentration in suspension C_{sus}, microbial protein concentration C_X, phenanthrene consumption (degradation flux) $dm^{-1}dt^{-1}$ and microbial growth rate constant $dX\,dt^{-1}X^{-1}$. Coefficient of determination R^2, root mean squared error *RMSE* and mean absolute error *MAE* for the different experiments (nominal initial phenanthrene concentration C_0).

Experiment	C_{sus} mod vs. meas			C_X mod vs. meas			$dm_M\,dt^{-1}$ mod vs. meas			$dX\,dt^{-1}X^{-1}$ mod vs. meas		
	R^2 (-)	*RMSE* (mg L^{-1})	*MAE* (mg L^{-1})	R^2 (-)	*RMSE* (mg L^{-1})	*MAE* (mg L^{-1})	R^2 (-)	*RMSE* (µg d^{-1})	*MAE* (µg d^{-1})	R^2 (-)	*RMSE* (d^{-1})	*MAE* (d^{-1})
Novosphingobium pentaromativorans												
C_0 = 10 mg L^{-1}	0.994	0.25	0.12	0.540	0.31	0.22	0.896	4.53	2.29	0.006	0.17	0.10
C_0 = 25 mg L^{-1}	0.999	0.32	0.23	0.871	0.82	0.68	0.967	3.68	2.05	0.794	0.44	0.31
C_0 = 50 mg L^{-1}	0.994	2.02	1.58	0.953	0.46	0.33	0.861	20.7	12.5	0.658	0.96	0.64
C_0 = 100 mg L^{-1}	0.989	6.10	3.96	0.883	1.36	1.18	0.880	21.7	14.4	0.625	1.09	0.63
C_0 = 200 mg L^{-1}	0.990	14.06	9.33	0.903	2.65	2.22	0.624	69.8	39.4	0.838	1.30	0.77
C_0 = 400 mg L^{-1}	0.996	11.80	7.44	0.848	6.48	4.49	0.962	39.8	24.8	0.661	1.32	0.84
Sphingomonas sp. EPA505												
C_0 = 10 mg L^{-1}	0.908	1.04	0.62	0.033	0.92	0.80	0.433	16.1	10.1	0.001	1.07	0.80
C_0 = 25 mg L^{-1}	0.972	1.93	1.31	0.203	1.55	0.87	0.885	16.0	10.0	0.263	2.14	1.27
C_0 = 50 mg L^{-1}	0.985	2.70	1.88	0.225	4.00	3.11	0.961	20.5	13.2	0.578	2.06	1.07
C_0 = 100 mg L^{-1}	0.977	7.88	5.00	0.819	3.04	2.46	0.765	57.4	31.4	0.387	1.65	0.88
C_0 = 200 mg L^{-1}	0.998	5.18	3.52	0.127	11.66	9.99	0.989	14.2	10.5	0.600	1.64	0.85
C_0 = 400 mg L^{-1}	0.994	11.63	7.47	0.730	6.41	4.94	0.957	29.9	23.0	0.793	1.76	1.00
Sphingobium yanoikuyae												
C_0 = 10 mg L^{-1}	0.996	0.15	0.13	0.221	3.04	2.43	0.962	1.88	1.08	0.459	1.53	0.91
C_0 = 25 mg L^{-1}	0.982	0.92	0.49	0.186	4.31	3.51	0.789	14.2	8.33	0.728	2.96	1.74
C_0 = 50 mg L^{-1}	0.904	5.39	3.14	0.234	12.89	11.07	0.566	57.8	36.8	0.842	3.40	2.03
C_0 = 100 mg L^{-1}	0.980	6.65	3.33	0.033	7.51	4.58	0.727	44.4	24.4	0.582	2.47	1.23
C_0 = 200 mg L^{-1}	0.991	8.74	5.41	0.687	3.70	2.92	0.926	35.2	22.3	0.864	1.84	0.98
C_0 = 400 mg L^{-1}	0.986	23.03	15.50	0.863	4.95	3.25	0.620	115	81.5	0.889	2.12	1.16

Discussion of dissolution kinetics and lag phases

The factor f_A is an additional fit parameter that was required due to changes of the dissolution and metabolism kinetics during the experiment (Table SI1); $f_{A,0}$ increases or decreases the initial substance flux, and the exponent k_{agg}, which describes the decrease of dissolution flux over time.

At low initial concentrations (up to 50 mg L^{-1}) the dissolution ($f_{A,0}$ and subsequently also the loss) of PHE was fastest in the experiments with *Sphingomonas* sp. EPA505. The initial $f_{A,0}$ was also high with *Sphingobium yanoikuyae*, but the dissolution in the experiments with that strain declined rapidly within one day, as described by a high rate k_{agg}, and PHE was still present at the end of the experiments (t = 6 d) with initial concentration 10 mg L^{-1} (final 0.32 mg L^{-1}) and 25 mg L^{-1} (final 0.5 mg L^{-1}). A pronounced lag phase with delayed metabolism occurred for low substrate concentrations. This lag lasted for about one day and was probably because the bacteria were transferred from a PHE solution of about 100 mg L^{-1} into the experimental vessels and can have stored substrate in the cells. *Sphingobium yanoikuyae* showed the most pronounced lag phase ($f_{inh,corr}$ in Table SI1). *Novosphingobium pentaromativorans* also had a considerable lag phase at both initial 10 and 25 mg L^{-1}, and *Sphingomonas sp.* had a minor lag at 10 mg L^{-1} initial concentration.

At higher initial concentrations (100, 200 and 400 mg L^{-1}), the dissolution kinetics of strain *Sphingobium yanoikuyae* was high during the whole experiment, whereas *Sphingomonas* sp. EPA505 showed a declining kinetics (k_{agg} 0.5, 0.5 and 0.3 d^{-1}, Table SI1) and also had the highest residual concentrations at the end of the experiment (t = 12 d). This can also indicate toxic effects or inhibition. The dissolution fit parameters obtained for the experiments with *Novosphingobium pentaromativorans* were more uniform, with decline at all concentrations.

The dissolution factor f_A can be interpreted as describing more than one process. On the one hand, the (uncontrolled) dissolution kinetics of crystals: the dissolution can change over time, as described, because the number of crystals in the system, and thus the surface area available for dissolution, decreases; this may or may not limit the availability of substrate. On the other hand, the factor f_A might also be affected by the bacteria themselves: either because these increase or decrease the dissolution kinetics, or because they take up less PHE with time, which may indicate bottlenecks in the metabolic flow or inhibition by toxic metabolites. We could not use two separate parameters to describe the effects of dissolution kinetics and inhibition, because this would give ambiguous fits.

In degradation experiments with polychlorinated biphenyls (PCB), a highly significant positive linear correlation between fitted maximum removal rate v_{max} and microbial decay rate constant b was found in experiments under resting cell (no-growth) conditions.[4] Similarly, in the present study, degradation fluxes lower than expected might result from such inhibitory effects.

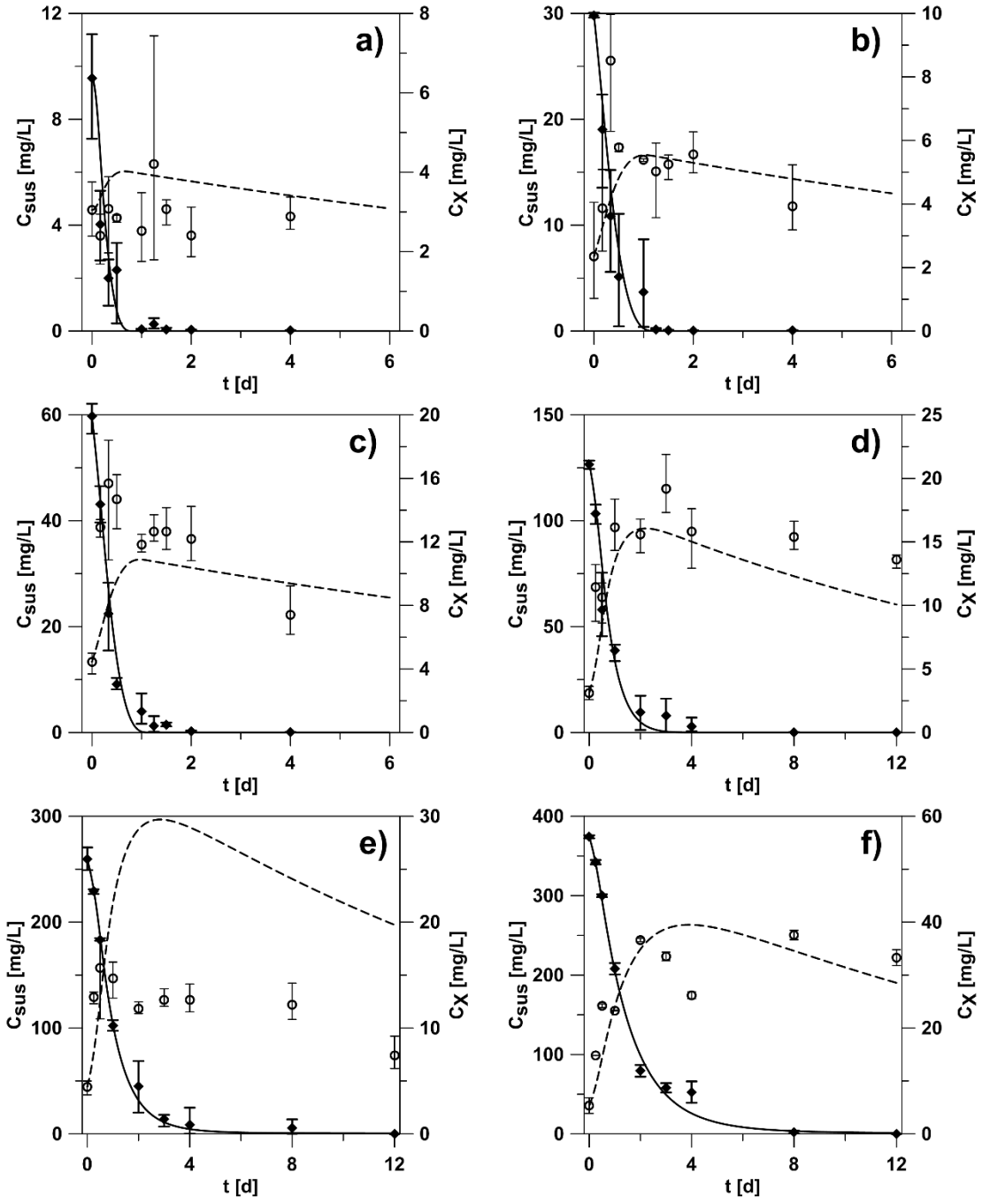

Figure SI 1. Experiments with *Sphingomonas* sp. EPA505: phenanthrene concentration in suspension C_{Sus} (---♦---) and protein concentration C_X (- -○- -), measured (data points) versus modelled (curves). Experiments with a) C_0 = 10 mg L^{-1}, b) C_0 = 25 mg L^{-1}, c) C_0 = 50 mg L^{-1}, d) C_0 = 100 mg L^{-1}, e) C_0 = 200 mg L^{-1}, f) C_0 = 400 mg L^{-1}. Error bars indicate minimum and maximum (3 replicates).

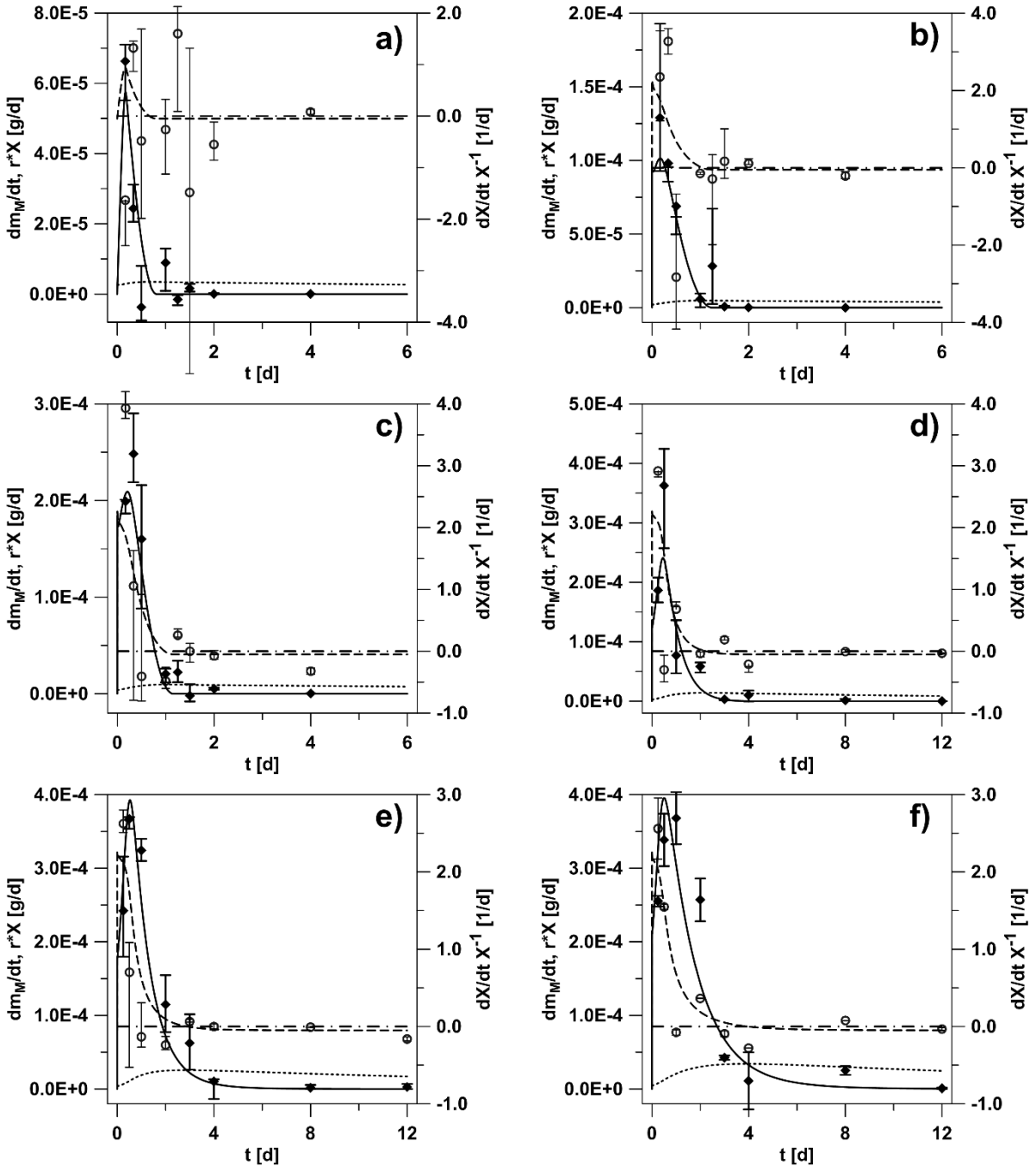

Figure SI 2. Experiments with *Sphingomonas* sp. EPA505: phenanthrene consumption (degradation velocity) dm_M/dt, (---♦---), microbial growth rate constant dX/dt/X (- -○- -) and maintenance r^*X (……) versus time, measured (data points) vs. modelled (curves). Point-dashed lines (-.-.-) indicates no-growth (dX/dt/X = 0). Experiments with a) C_0 = 10 mg L^{-1}, b) C_0 = 25 mg L^{-1}, c) C_0 = 50 mg L^{-1}, d) C_0 = 100 mg L^{-1}, e) C_0 = 200 mg L^{-1}, f) C_0 = 400 mg L^{-1}. Error bars indicate minimum and maximum (3 replicates).

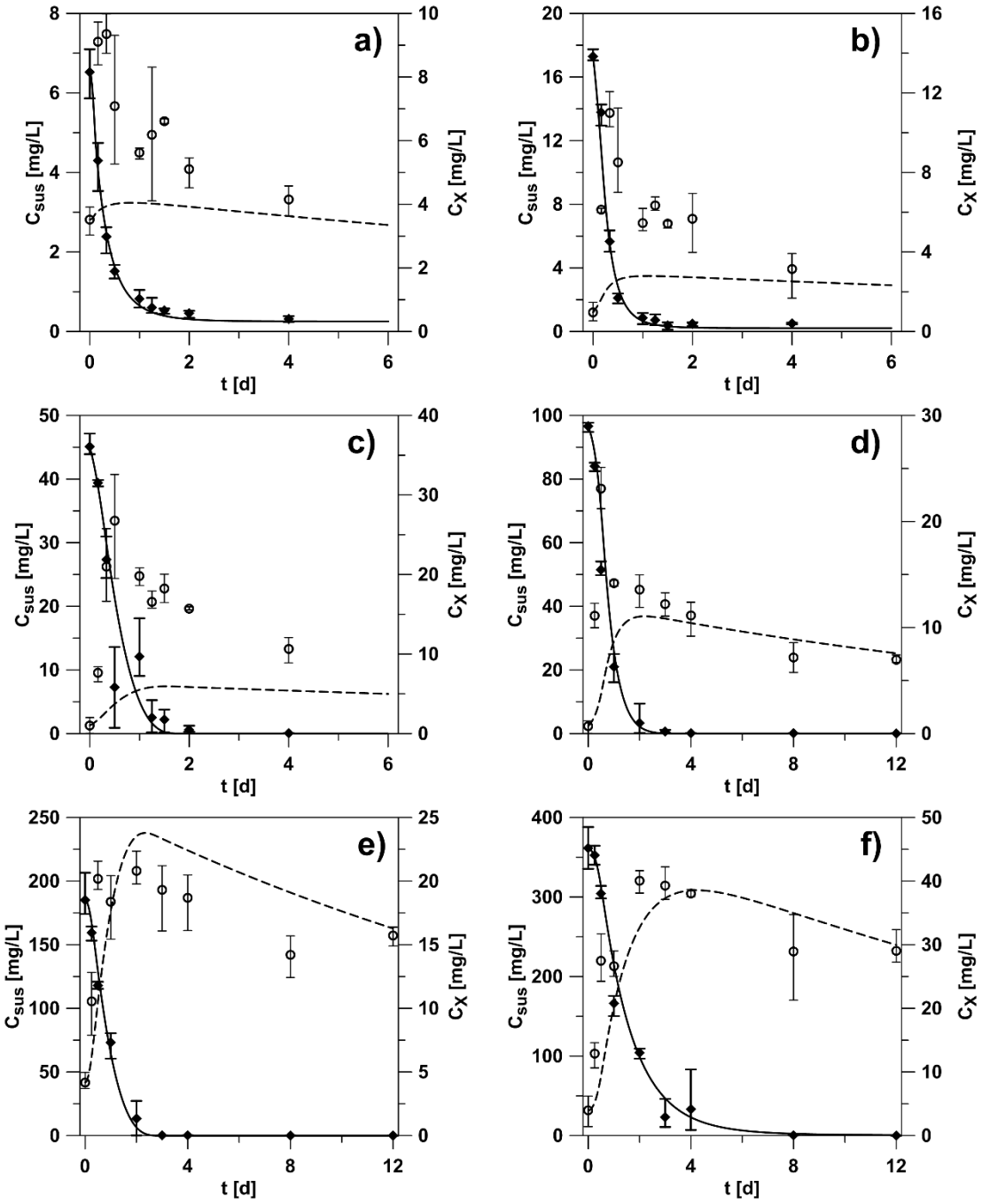

Figure SI 3. Experiments with *Sphingobium yanoikuyae*: phenanthrene concentration in suspension C_{Sus} (---♦---) and protein concentration C_X (- -○- -), measured (data points) versus modelled (curves). Experiments with a) C_0 = 10 mg L^{-1}, b) C_0 = 25 mg L^{-1}, c) C_0 = 50 mg L^{-1}, d) C_0 = 100 mg L^{-1}, e) C_0 = 200 mg L^{-1}, f) C_0 = 400 mg L^{-1}. Error bars indicate minimum and maximum (3 replicates).

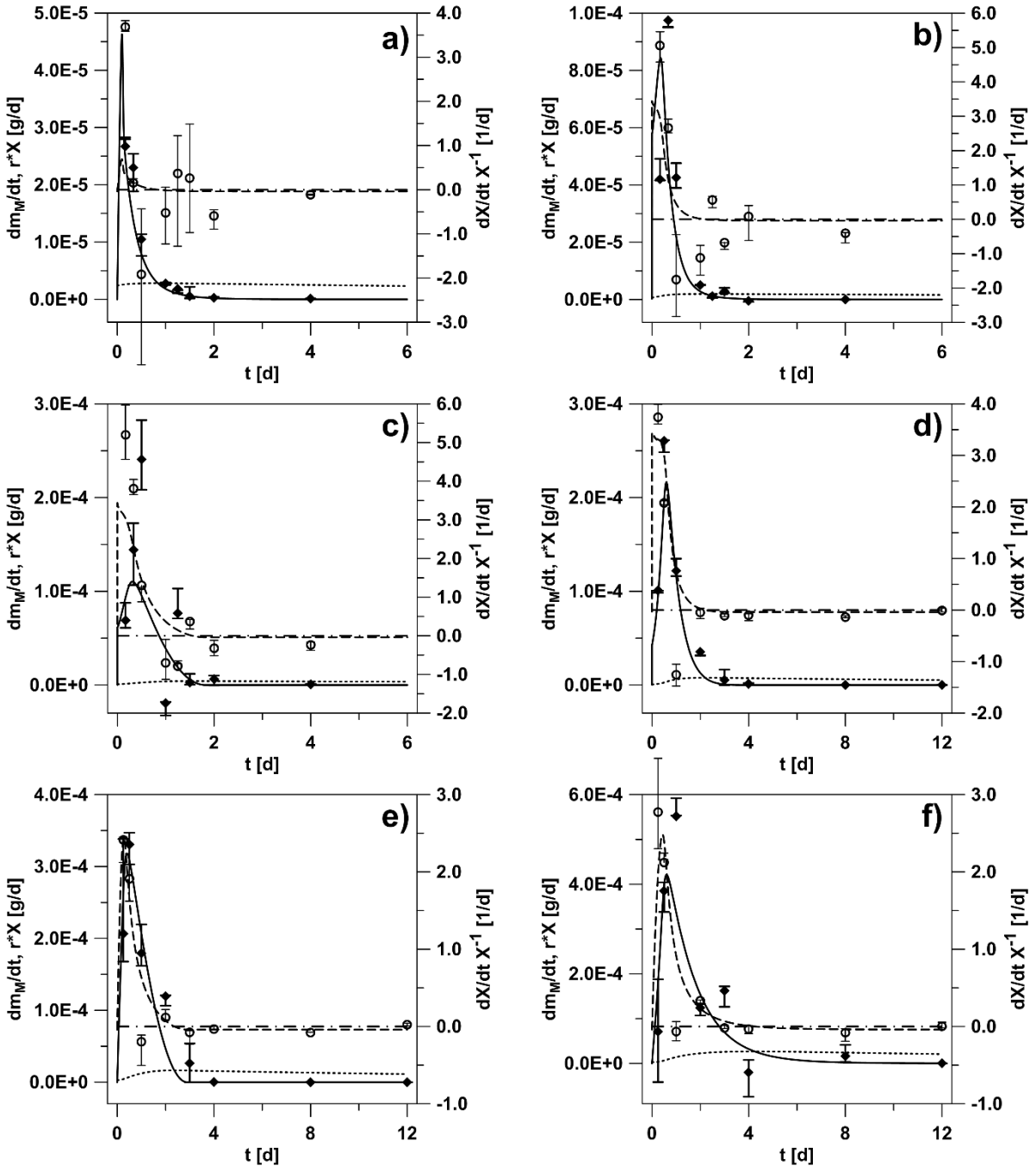

Figure SI 4. Experiments with *Sphingobium yanoikuyae*: phenanthrene consumption (degradation velocity) dm_M/dt, (---♦---), microbial growth rate constant dX/dt/X (- -○- -) and maintenance r^*X (……) versus time, measured (data points) vs. modelled (curves). Point-dashed lines (-.-.-) indicates no-growth (dX/dt/X = 0). Experiments with a) C_0 = 10 mg L^{-1}, b) C_0 = 25 mg L^{-1}, c) C_0 = 50 mg L^{-1}, d) C_0 = 100 mg L^{-1}, e) C_0 = 200 mg L^{-1}, f) C_0 = 400 mg L^{-1}. Error bars indicate minimum and maximum (3 replicates).

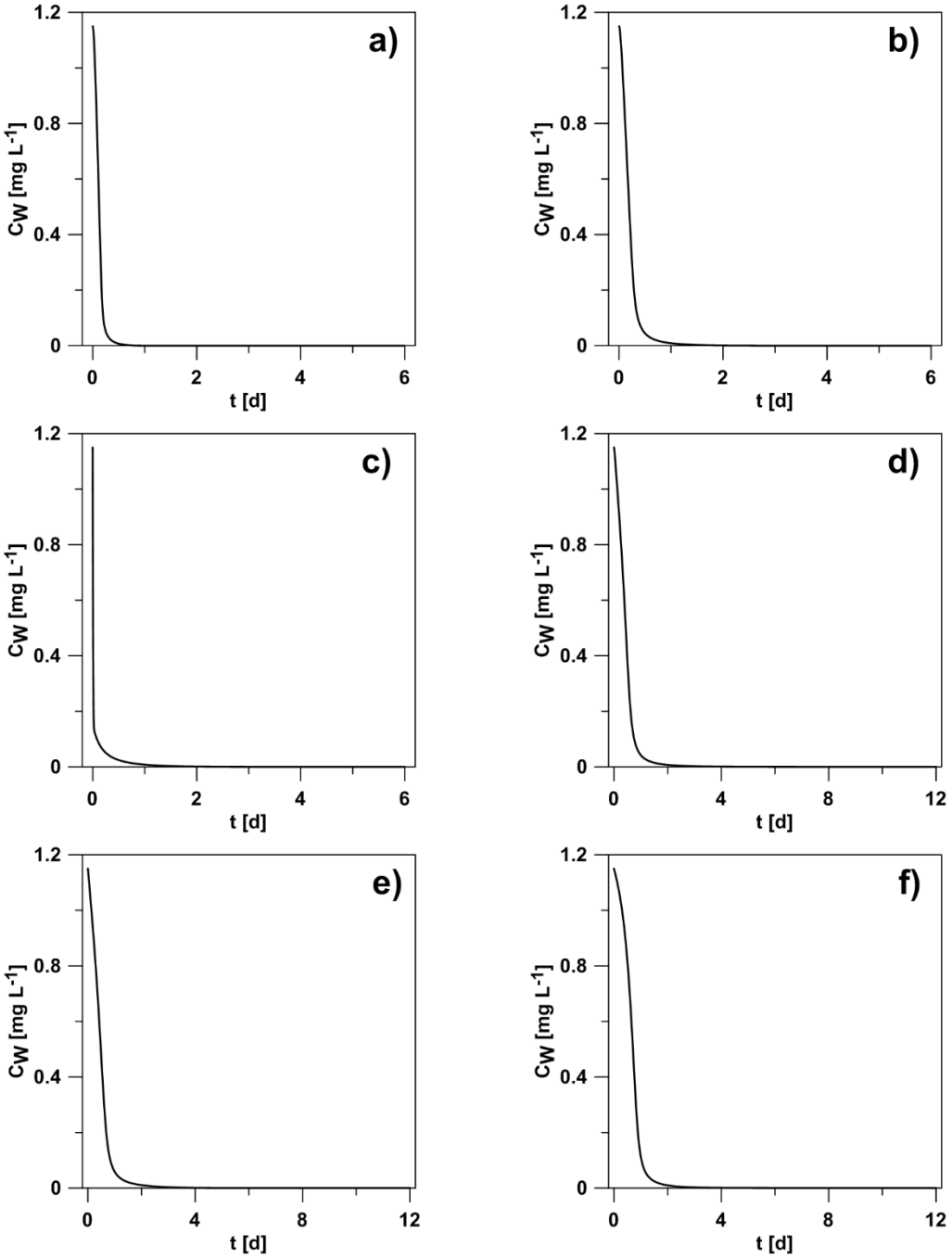

Figure SI 5. Modelled freely dissolved concentration C_W vs. time, with strain *Novosphingobium pentaromativorans*. Experiments with nominal initial phenanthrene concentrations: a) C_0 = 10 mg L^{-1}, b) C_0 = 25 mg L^{-1}, c) C_0 = 50 mg L^{-1}, d) C_0 = 100 mg L^{-1}, e) C_0 = 200 mg L^{-1}, f) C_0 = 400 mg L^{-1}.

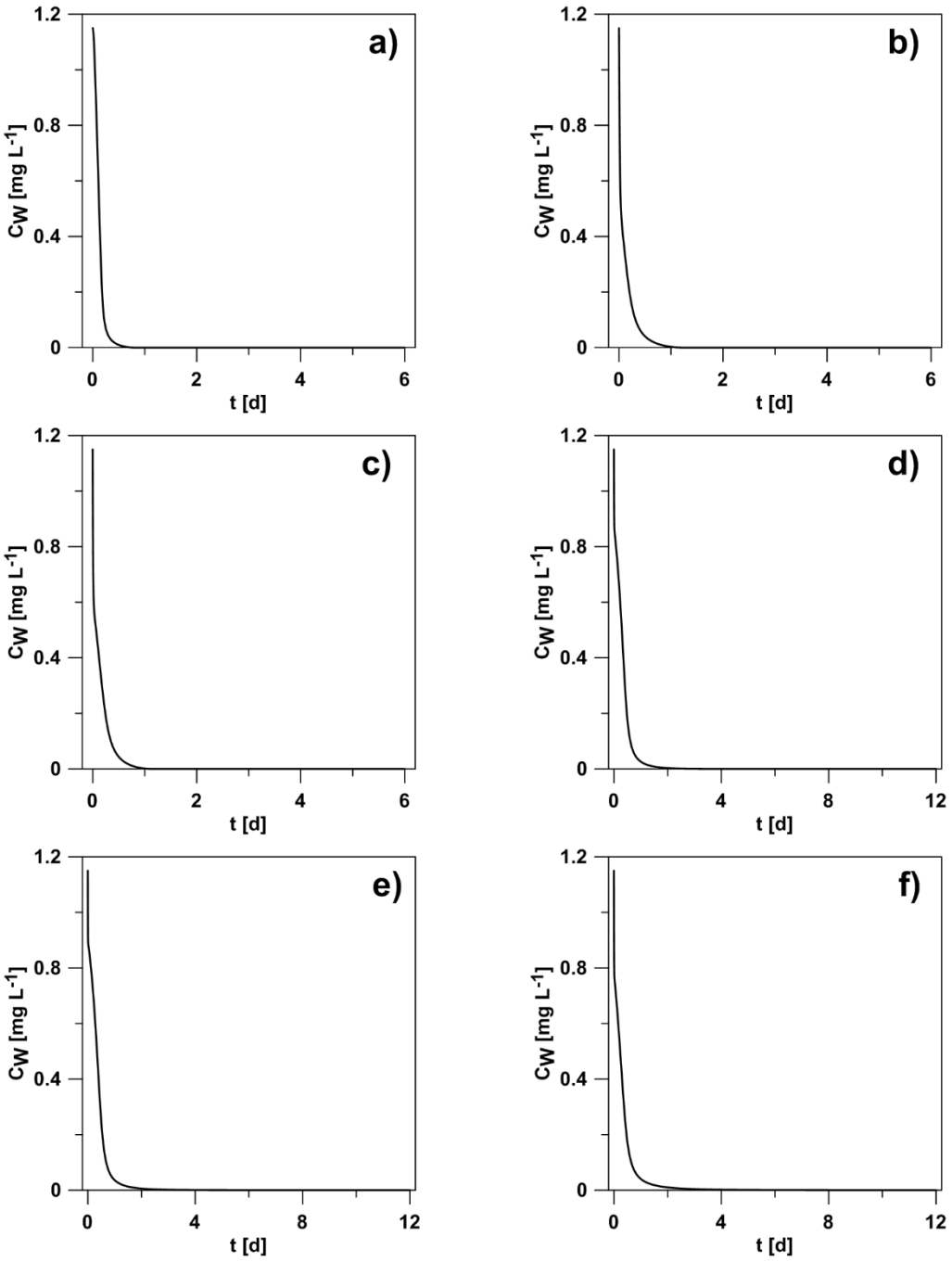

Figure SI 6. Modelled freely dissolved concentration C_W vs. time, with strain *Sphingomonas* sp. EPA505. Experiments with nominal initial phenanthrene concentrations: a) C_0 = 10 mg L^{-1}, b) C_0 = 25 mg L^{-1}, c) C_0 = 50 mg L^{-1}, d) C_0 = 100 mg L^{-1}, e) C_0 = 200 mg L^{-1}, f) C_0 = 400 mg L^{-1}.

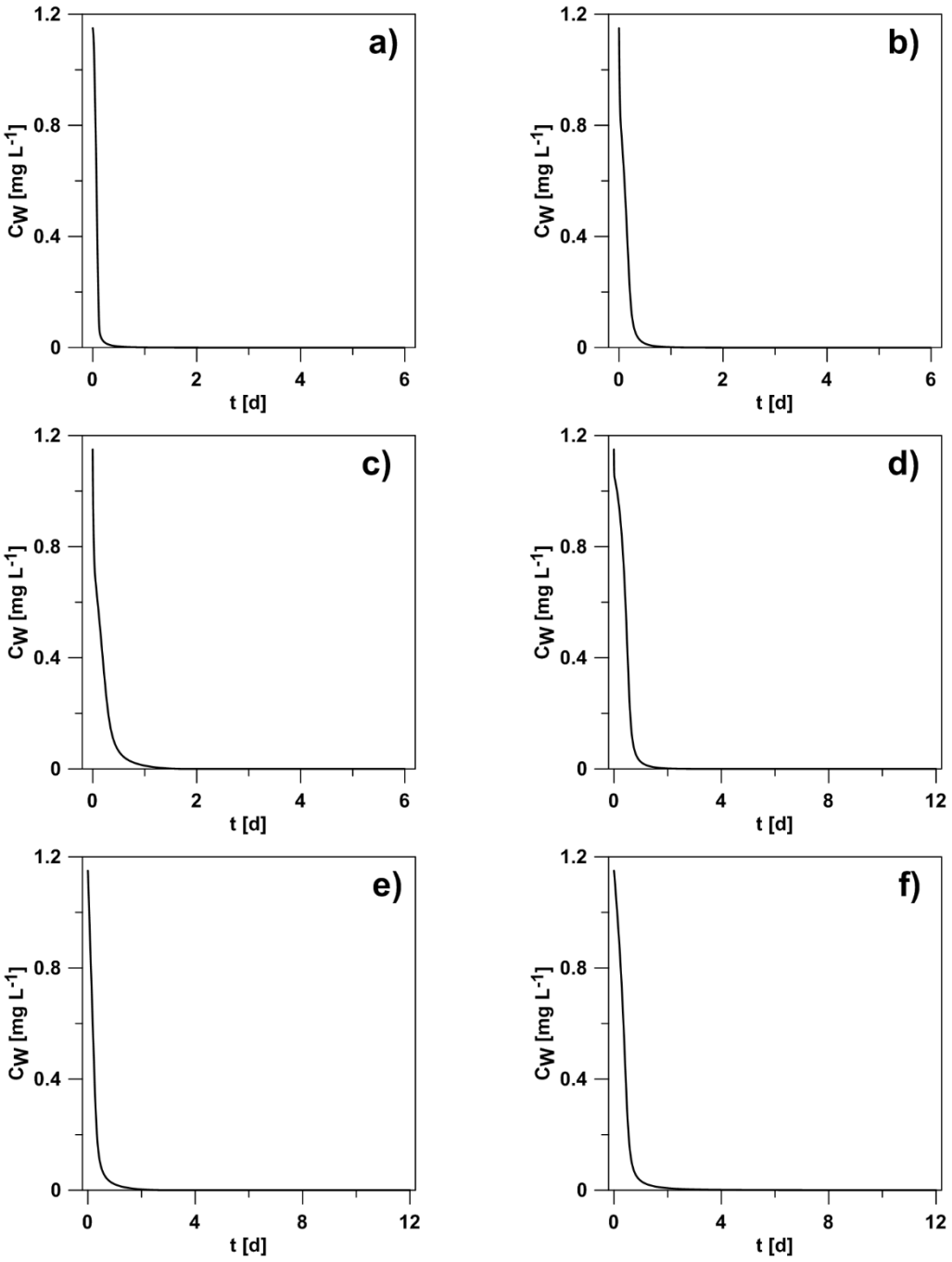

Figure SI 7. Modelled freely dissolved concentration C_W vs. time, with strain *Sphingobium yanoikuyae*. Experiments with nominal initial phenanthrene concentrations: a) C_0 = 10 mg L^{-1}, b) C_0 = 25 mg L^{-1}, c) C_0 = 50 mg L^{-1}, d) C_0 = 100 mg L^{-1}, e) C_0 = 200 mg L^{-1}, f) C_0 = 400 mg L^{-1}.

References:

(1) Droop, M. R. Some thoughts on nutrient limitation in algae. *Journal of Phycology* **1973,** *9*, 264-272.

(2) Collins, C. D. Formulation and validation of a mathematical model of phytoplankton growth. *Ecology* **1980,** *61*, 639-649.

(3) Rabouille, S.; Staal, M.; Stal, L. J.; Soetaert, K. Modeling the dynamic regulation of nitrogen fixation in the cyanobacterium *Trichodesmium* sp. *Applied and Environmental Microbiology* **2006,** *72*, 3217-3227.

(4) Rein, A.; Fernqvist, M. M.; Mayer, P.; Trapp, S.; Bittens, M.; Karlson, U. G. Degradation of PCB congeners by bacterial strains. *Environmental Biotechnology* **2007,** *77*, 469-481.