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A general model for multiple substrate biodegradation. Application to co-metabolism of structurally non-analogous compounds

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Abstract

The availability of multiple carbon/energy sources, as is common in wastewater treatment plants, often enhances the biodegradation of recalcitrant compounds. In this paper, we classify and model different modes of multiple substrate utilization in a systematic way, using the concept of synthesizing unit. According to this concept, substrates can be substitutable or complementary; their uptake (or processing) can be sequential or parallel. We show how the different modes of multiple substrate interaction can be described by a single general model. From the general model, we derive simple expressions for co-metabolism of substrates that are not structurally analogous. Both the general and the specific co-metabolism model have the advantage that they can be used in combination with any microbial growth model. To test the co-metabolism model's realism, we confront it with experimental data. The results attained with the co-metabolism model support that the general model constitutes a useful framework for modeling aspects of multiple substrate utilization.

Keywords: Bioremediation; Multiple-substrate degradation; Co-metabolite; Co-metabolism; Dynamic Energy Budgets; Synthesizing Unit

1. Introduction

For microbial growth, the relevant features of an ecosystem include its physico-chemical conditions and the type and concentration of the available resources. Although the availability of one primary resource often suffices to ensure growth of a microbial population, many species are able to use more than one carbon source simultaneously. This phenomenon is known as co-utilization. Microorganisms can attain a considerable growth rate by using multiple carbon sources simultaneously, even when each of them is present in a very low concentration [1]. As it is the amount of biomass that determines nutrient requirements, co-utilization influences the biodegradation rates of the involved substrates. Co-utilization can thus enhance biodegradation simply by increasing the biomass of the degraders.

Simultaneous biodegradation of substrates is not only important for microorganisms, but also for bioremediation of polluted ecosystems. Our environment is polluted with many 'man-made' chemicals, but fortunately microorganisms are able to transform or even degrade many of them. Sometimes a contaminant is degraded because it serves as an (additional) energy source. We then deal with proper co-utilization of the contaminant. Yet, a contaminant can also be fortuitously degraded.

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Nomenclature

S_*	concentration of compound $*$ ($\#l^{-3}$)
j_*	specific arrival rate of compound $*$ ($\#\#^{-1}\ t^{-1}$)
j_*^+	specific biodegradation rate of compound $*$ ($\#\#^{-1}\ t^{-1}$)
j'_*	scaled arrival rate of compound $*$: $j'_* = \rho_* j_*$ ($\#\#^{-1}\ t^{-1}$)
j''_{*_1}	scaled arrival rate of compound $*$: $j''_* = \rho_{*_1 *_2} j_{*_1}$ ($\#\#^{-1} t^{-1}$)
k_*	handling rate for compound $*$ (t^{-1})
K_*	saturation coefficient of compound $*$ ($\#l^{-3}$)
$y_{*_1 *_2}$	stoichiometric coefficient (coupler): compound 1 ($*_1$) needed per compound 2 ($*_2$) formed ($\#\#^{-1}$)
ρ_*	binding probability of compound $*$ to SU (—)
$\rho_{*_1 *_2}$	binding probability of compound $*_1$ to SU–$*_2$ complex (—)
$\theta_{*_1 *_2}$	fraction of SUs occupied by substrates $*_1$ and $*_2$ (—)

The following symbols are used for the dimensions: —, no dimension; t, time; l, length; #, amount (C-mol or mass).

The presence of easily degradable carbon sources can enhance the biodegradation of more persistent chemicals. This is best illustrated by the process of co-metabolism. Such a variation on biodegradation has been defined as fortuitous transformation of a contaminant that cannot serve as primary energy source for the microorganisms [2,3]. Although co-metabolized compounds can be a burden to the cell, the process is important as it determines the fate of chemicals in the environment [4,5]. For instance, co-metabolic transformations can produce compounds which are readily degraded by other environmental microorganisms. Such a commensal relationship between microorganisms has been observed in the degradation of cyclohexane. *Mycobacterium vaccae*, growing on propane, transforms cyclohexane to cyclohexanone, which serves as a growth substrate for another species [6]. Quantitative knowledge of co-metabolism plays an important role in, for instance, bioremediation of chemically polluted soils by addition of readily metabolizable substrates.

As explained above, multiple substrate utilization is a very important process. Particular cases of multiple substrate utilization, such as multiple nutrient limitation and co-metabolism, have been described by different modeling approaches, which proceed much in isolation of each other. The aim of this paper is to provide a general model that embraces the different aspects of multiple substrate utilization.

This paper is organized as follows. In the next section, we develop a mathematical framework for dual substrate utilization in which substrates play a symmetric role. The resulting kinetic equations are based on general mechanistic arguments. They are, therefore, suited to accommodate features corresponding to specific types of multiple substrate utilization. Thanks to this property, adequate assumptions on the parameter values naturally lead to kinetic equations for four well-known modes of interaction between two substrates. In Section 3, we further illustrate the advantages of our general framework by using it to derive a model for co-metabolism of structurally non-analogous substrates (i.e., co-metabolism without competitive inhibition). We show the results of fitting the obtained co-metabolism model to experimental data [7,8] and compare the model with existing approaches to co-metabolism. In Section 4, we discuss the advantages and limitations of both our general framework and the co-metabolism model.

2. Model framework

As already emphasized above, the relationship between biodegradation and biomass growth is important. To account for this relationship, we devised a model for multiple substrate assimilation that is suited to be combined with any microbial growth model. The simplest microbial growth model takes the growth rate proportional to the substrate consumption rate: the well-known Monod model. It is at the basis of a series of models of increasing complexity and realism. This series includes models that account for maintenance only [9–11], for reserves only [12], and for both maintenance and reserves (DEB [13]). The latter has been recently extended to include growth of microbial flocculles [14]. For a critical overview of existing microbial growth models and mixed substrate utilization, we refer the reader to [15]. The chemical composition of the biomass is constant in the Monod and Marr–Pirt models, whereas it depends on growth conditions in the Droop and DEB models. In Section 3.1, we exemplify how the Monod model can be used in combination with our biodegradation model.

We seek to quantitatively characterize the degradation of compounds in situations in which multiple substrate biodegradation takes place. In this paper, we focus on microorganisms degrading two substrates, A and B. The resulting mathematical model can be analogously formulated for an arbitrary number of substrates,

however. We view a microorganism as a 'generalized enzyme' that transforms substrates A and B into a product C. The kinetics of the generalized enzyme then determines the expressions for the sought (biomass) specific biodegradation rates of substrates A and B, denoted by j_A^+ and j_B^+. The interpretation of the product C as well as the relation between the biodegradation rates and the microbial growth rate depend on what the microorganisms actually do with the degraded compounds. Firstly, they do not necessarily transform both compounds into new biomass. For instance, substrate B could be a fortuitously degraded no energy supplying contaminant. As long as B has no effect on growth, the microbial growth rate only depends on the A consumption rate. Secondly, as said above, the fate of assimilated substrates varies from one microbial growth model to another. According to the Monod model, any assimilated substrate molecule results in new biomass. The production rate (j_C) is then equal to the growth rate. Alternatively, according to the DEB model [13], assimilated substrates are first transformed into reserves.

To characterize the transformation of multiple substrates into a product C, the concept of synthesizing unit (SU) is particularly suited. Indeed, the SU-kinetics can be analytically generalized for an arbitrary number of substrates [16]. An SU can be defined as a generalized enzyme that follows classic association–dissociation kinetics with two modifications [13,16]: (i) production rates relate to arrival rates of substrates at the SU, and (ii) the dissociation rates between substrates and SUs are negligibly small. The translation of SU-kinetics into equations leads to an attractively simple mathematical model that can be applied in quite complex situations, ranging from microbial growth to population dynamics. Among them are: nutrient-limited growth of *Daphnia* in a closed system with phosphorus-limited algae [17]; multiple nutrient limitation of algal growth [13]; photosynthesis and photo-respiration [13]; stoichiometric constraints on population dynamics [18]; quantitative steps in symbiogenesis [19]; mixotrophy [20]; microbial adaptation to changing availability of substrates [21]; and multiple substrate utilization and co-metabolism (this paper).

As SU-kinetics is based on arrival rates rather than on concentrations, the versatility of the SU concept becomes evident in spatially structured (or heterogenous) environments, like the interior of a cell, where the concept of concentration is difficult to apply [22]. In well-mixed environments, where the concept of concentration does apply, the arrival rates are proportional to concentrations on the basis of the law of mass action and the link with classic enzyme kinetics is restored. The SU-based expression for single substrate uptake then simplifies to the well-known Michaelis–Menten kinetics.

During the transformation of one substrate molecule A into product C by an SU it is possible to define the following stages:

1. a substrate molecule arrives at the SU;
2. if the SU has already a bound substrate, the arriving molecule is rejected, whereas if the SU is not occupied, the arriving molecule has a certain probability $0 \leqslant \rho \leqslant 1$ to bind to the SU;
3. the SU transforms the substrate molecule into product;
4. the product is released and the SU can bind substrate again.

When an SU transforms two substrates into product, this scheme complicates somewhat because interaction between the substrates can occur. For instance, substrate A could inhibit the biodegradation of substrate B. This means that B has a larger binding probability when it arrives at a free SU than when it arrives at an SU–A complex. Substrate interaction in multiple substrate uptake is the subject of the next section. Thereafter, we will show how the different modes of interaction can be systematically modeled using SU-kinetics.

2.1. Four types of dual substrate degradation

Degradation processes can be classified according to the relative role of substrates in product formation and to their interaction during processing. With regard to their relative role in product formation, simultaneously degraded substrates can be substitutable or complementary. Substrates are called substitutable when they can be separately transformed into product C, that is $y_{AC}A \rightarrow C$ and $y_{BC}B \rightarrow C$. The symbol y denotes a coupler or stoichiometric coefficient. So, y_{CA} represents the amount of C formed per amount of A and y_{AC} the amount of A degraded per amount of C formed ($y_{AC} = y_{CA}^{-1}$). Simultaneously degraded substrates are called complementary when both are required to produce C, that is $y_{AC}A + y_{BC}B \rightarrow C$. The absence of one complementary substrate prevents the degradation of the other, since both substrates must bind to the SU before any product is released. Complementary degradation occurs, for example, if both oxygen and a carbon/energy source are growth limiting.

Both substitutable and complementary substrates can be classified according to the presence or absence of interaction at the substrate binding/processing level. For two substrates, this results in four possible modes of interaction, which we refer to as substitutable-sequential, substitutable-parallel, complementary-sequential, and complementary-parallel. The reaction diagrams for these possible modes of degradation are shown in Fig. 1.

For complementary substrates, interaction in the binding process means that one of the substrates can

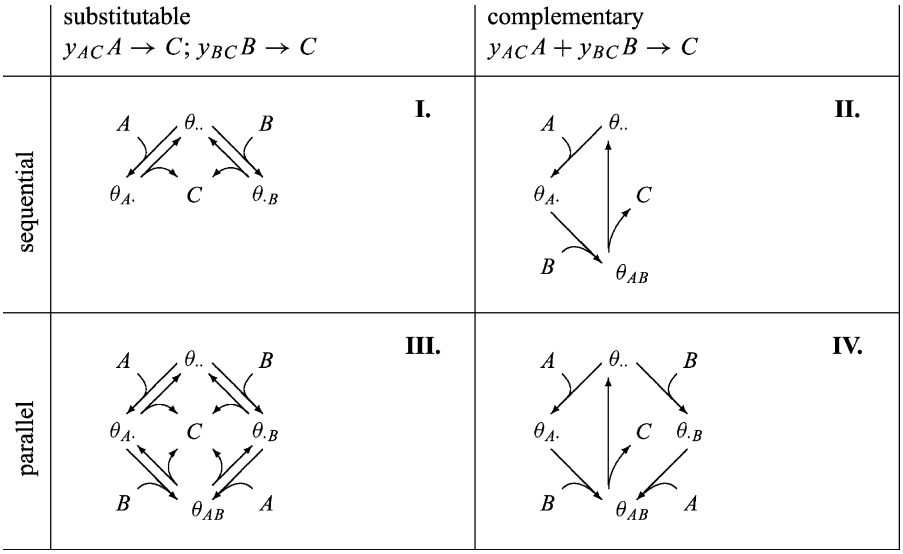

Fig. 1. Modes of transformation of two substrates A and B into product C. The coefficient y_{AC} represents the amount of A consumed per amount C formed and, likewise, y_{BC} represents the amount of B degraded per amount C formed. The four panels show the state transitions of the SUs during the four different modes of transformation. The symbols $\theta_{..}$, $\theta_{A.}$, $\theta_{.B}$, and θ_{AB} represent the fraction of SUs in a particular binding state. A dot means absence of substrate, so $\theta_{..}$ represents the fraction of free SUs. Similarly, θ_{AB} represents the fraction of SUs with both substrates A and B bound. In panel I, for example, a free SU ($\theta_{..}$) may bind either substrate A or B giving $\theta_{A.}$ or $\theta_{.B}$, respectively. These SUs can return to the state $\theta_{..}$ by releasing product C. According to the concept of SU, substrates are either substitutable or complementary; binding can be either sequential or parallel.

only bind to an SU if it is already bound to the other substrate. In diagram II (Fig. 1), for example, substrate B only binds to the SU–A complex. This is called complementary-sequential degradation. If no interaction between the complementary substrates occurs in the binding process, we deal with complementary-parallel degradation. Occurrence or absence of interaction between complementary substrates can be characterized in terms of binding orders. If the binding order of the substrates is relevant, complementary-sequential degradation results. In diagram II (Fig. 1), for example, we assumed that substrate A must first bind to the SU. The mathematical expression for this mechanism is simple and has interesting mathematical properties [13, p. 45]. Its practical interest is limited as the binding order is usually not important and, thus, complementary-parallel degradation takes place. The corresponding model has been used to satisfactorily describe dual substrate limited growth of the haptophyte *Pavlova lutheri* [13, p. 170], where phosphorus and vitamin B_{12} were the limiting nutrients.

For substitutable substrates, interaction in the binding process means that a substrate of one type cannot bind to the SU while it is processing a substrate of the other type. An increase in the abundance of only one substrate decreases the biodegradation rate of the other. We refer to this situation as substitutable-sequential degradation. Indeed, it is equivalent to competitive interaction, which is often due to competition of structurally analogous substrates for the same binding site [23].

If two substitutable substrates do not interfere with each other in the binding process, we deal with substitutable-parallel degradation. Substitutable-parallel degradation occurs, for instance, when two substrates that support growth have a negligible interaction in the cell's metabolism. This results in additive uptake/growth models. Hanegraaf [24] modeled the simultaneous maltose and glucose utilization by *Saccharomyces cerevisiae* in this parallel way. The uptake of one substrate does not affect the uptake of the other substitutable substrate as long as their binding probabilities are independent. Although the uptake processes hardly interact directly due to the use of different carriers, the subsequent processing shares common machinery. This can introduce some properties of sequential processing. We will deal with this kind of 'mixed degradation' in the next section.

2.2. Modeling mixed degradation

In this section, we deduce a general model that accounts for the four types of dual substrate degradation explained above. The reaction diagram described by this model is depicted in Fig. 2. It is this general type of degradation that we referred above as mixed degradation. To introduce this concept, let us consider a

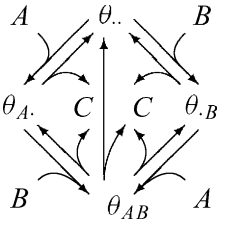

Fig. 2. Mixed degradation. Notation as in Fig. 1. This mixed diagram can be reduced to any of the diagrams shown in Fig. 1 by deleting one or more arrows. In mathematical terms, this implies that once expressions describing mixed degradation are known, expressions for any of the four types of degradation can be obtained by choosing appropriate conditions on the model parameters.

microorganism that assimilates both a carbon source (A) and a nitrogen source, for example an amino acid (B). If the microorganism is able to synthesize the amino acid de novo, a mixed type of assimilation results. When the amino acid is available from the medium, the microorganism uses this source, which results in an enhanced yield. The yield on A and B together exceeds the yield on A or B.

Kooijman [16] has obtained a formulation for SU-kinetics that involves explicit stoichiometry (where the stoichiometric coefficients y are rational numbers of reacting molecules) in a stochastic setting. Because the formation of biomass cannot be specified at the molecular level, we use a simple deterministic approximation with implicit stoichiometry. Like in classic enzyme kinetics, this leads to expressions for the change in the fraction of free SUs and in the fractions of SUs that are bound to substrate A only, to substrate B only, or to both substrates. As can be seen from Fig. 2, in mixed assimilation we deal with an SU that carries out three transformations $y_{AC}A \to C$, $y_{BC}B \to C$, and $y'_{AC}A + y'_{BC}B \to y_{C+}C$. When a substrate molecule arrives at the SU, it has a probability ρ to bind to the SU. As explained in the previous section, this probability depends on the state of the SU. For substrate A, we have

ρ_A if A and B are not bound,
ρ_{AB} if A is not bound, but B is bound,
0 if A is bound

and for substrate B

ρ_B if B and A are not bound,
ρ_{BA} if B is not bound, but A is bound,
0 if B is bound.

After binding the substrates, the SU enters the production stage. The handling rates (k_*), and the stoichiometric coefficients (y_{**}) can differ for both substrates. If only A or only B is used to produce C, we have handling rates k_A and k_B, respectively. If both A and B are required, the handling rate of the SU is denoted by k. Moreover, the handling rate of A can be different when B is also bound to an SU and vice versa: k_{AB} is the handling rate of A when B is bound to the SU; similarly k_{BA} is the handling rate of B when A is bound. When the product has been released, the SU is ready to bind other substrate molecules and the cycle starts again.

The different SU-fractions change according to the following dynamics:

$$\begin{aligned}
\frac{\mathrm{d}}{\mathrm{d}t}\theta_{..} &= -(\rho_A j_A + \rho_B j_B)\theta_{..} + k_A\theta_{A\cdot} + k_B\theta_{\cdot B} + k\theta_{AB},\\
\frac{\mathrm{d}}{\mathrm{d}t}\theta_{A\cdot} &= \rho_A j_A\theta_{..} - (k_A + \rho_{BA}j_B)\theta_{A\cdot} + k_{BA}\theta_{AB},\\
\frac{\mathrm{d}}{\mathrm{d}t}\theta_{\cdot B} &= \rho_B j_B\theta_{..} - (k_B + \rho_{AB}j_A)\theta_{\cdot B} + k_{AB}\theta_{AB},\\
\frac{\mathrm{d}}{\mathrm{d}t}\theta_{AB} &= \rho_{BA}j_B\theta_{A\cdot} + \rho_{AB}j_A\theta_{\cdot B} - (k_{AB} + k_{BA} + k)\theta_{AB},\\
1 &= \theta_{..} + \theta_{A\cdot} + \theta_{\cdot B} + \theta_{AB},
\end{aligned} \tag{1}$$

where j_A and j_B represent the arrival rates of substrates A and B, respectively. $\theta_{..}$, $\theta_{A\cdot}$, $\theta_{\cdot B}$, and θ_{AB} denote the fraction of SUs present in a particular binding-state. For further details on the interpretation of the θ's, see Fig. 1.

2.2.1. Quasi steady-state mixed kinetics

If we assume quasi steady-state, we are able to solve system (1) analytically. The solution is most easily expressed in terms of the net arrival rates, which are defined as $j'_A = j_A\rho_A$, $j''_A = j_A\rho_{AB}$, $j'_B = j_B\rho_B$, and $j''_B = j_B\rho_{BA}$. The quasi-steady-state solution is then given by $\theta^{ss}_{..} = \Theta_{..}/\Theta_+$, $\theta^{ss}_{A\cdot} = \Theta_{A\cdot}/\Theta_+$, $\theta^{ss}_{\cdot B} = \Theta_{\cdot B}/\Theta_+$ and $\theta^{ss}_{AB} = \Theta_{AB}/\Theta_+$, where $\Theta_+ = \Theta_{..} + \Theta_{A\cdot} + \Theta_{\cdot B} + \Theta_{AB}$ and

$$\begin{aligned}
\Theta_{..} &= j''_A j''_B k + j''_A k_A(k + k_{BA}) + j''_B k_B(k + k_{AB}) + k_A k_B k_+,\\
\Theta_{A\cdot} &= j''_A(j'_+ k_{BA} + j'_A k) + j'_A k_B k_+,\\
\Theta_{\cdot B} &= j''_B(j'_+ k_{AB} + j'_B k) + j'_B k_A k_+,\\
\Theta_{AB} &= j''_A j''_B j'_+ + j''_A j'_B k_A + j''_B j'_A k_B
\end{aligned}$$

with $k_+ = k + k_{AB} + k_{BA}$ and $j'_+ = j'_A + j'_B$. The specific biodegradation rates (j^+_A and j^+_B) and the corresponding specific production rate (j_C) are then given by:

$$j^+_A = k_A\theta^{ss}_{A\cdot} + (k_{AB} + y'_{AC}k)\,\theta^{ss}_{AB}, \tag{2}$$

$$j^+_B = k_B\theta^{ss}_{\cdot B} + (k_{BA} + y'_{BC}k)\,\theta^{ss}_{AB}, \tag{3}$$

$$\begin{aligned}
j_C = {} & y_{CA}k_A\theta^{ss}_{A\cdot} + y_{CB}k_B\theta^{ss}_{\cdot B}\\
& + (y_{CA}k_{AB} + y_{CB}k_{BA} + y_{C+}k)\theta^{ss}_{AB}.
\end{aligned}$$

These general equations for mixed kinetics embrace the four modes of degradation shown in Fig. 1, since these are characterized by specific sets of conditions on the SU parameters. Substitution of any set of conditions into the general equations suffices to obtain an expression for the corresponding SU-kinetics. The four sets are discussed below.

- In substitutable-sequential degradation (Fig. 1, diagram I) each substitutable substrate can only bind to

a free SU. As explained above, this interaction between substrates is equivalent to competitive inhibition. In terms of SU-kinetics it means $\rho_{AB} = \rho_{BA} = 0$ and, thus $\theta_{AB} = 0$. Consequently, the handling rates k, k_{AB}, and k_{BA} are no longer relevant.

- In complementary-sequential degradation (Fig. 1, diagram II) both substrates are required to produce C and substrate B can only bind to the SU–A complex. The corresponding kinetics result from the general mixed kinetics by substituting $\rho_B = \rho_{AB} = 0$ and $k_A = k_{AB} = k_B = k_{BA} = 0$. The condition $\rho_B = 0$ implies $\theta_{.B} = 0$.
- Substitutable-parallel degradation (Fig. 1, diagram III) takes place when two substitutable substrates do not interfere during the binding process. The solution results from the general solution by substituting $\rho_A \neq 0$, $\rho_B \neq 0$, $\rho_{AB} \neq 0$, $\rho_{BA} \neq 0$ and $k = 0$. In addition, for simplicity, it can be assumed that $\rho_{AB} = \rho_A$, $\rho_{BA} = \rho_B$ and $k_{AB} = k_A$, $k_{BA} = k_B$.
- In complementary-parallel degradation (Fig. 1, diagram IV) the binding order of the complementary substrates is not relevant. Since both A and B are needed to produce C, $k_A = k_{AB} = k_B = k_{BA} = 0$. In addition, for simplicity, it can be assumed that $\rho_{AB} = \rho_A$, $\rho_{BA} = \rho_B$.

It is thus possible to distinguish between substitutable- and complementary-sequential degradation on the basis of binding probabilities only. For the former, which is also known as cross-competitive inhibition, $\rho_{AB} = \rho_{BA} = 0$ holds whereas for the latter, the condition $\rho_B = \rho_{AB} = 0$ holds. The four sets of conditions are summarized in Table 1. In well-mixed environments, it is reliable to assume that arrival rates of compounds are proportional to their concentrations. This proportionality can be incorporated straightforwardly into Eqs. (2) and (3) as will be exemplified in the next section.

Table 1
The general equations for mixed kinetics (Eq. (1)) embrace the four modes of degradation shown in Fig. 1 (The specific sets of conditions on the SU parameters are summarized below)

	substitutable $y_{AC}A \to C;\ y_{BC}B \to C$	complementary $y_{AC}A + y_{BC}B \to C$
sequential	**I.** $\rho_{AB} = 0$ $\rho_{BA} = 0$ $\theta_{AB} = 0$	**II.** $\rho_B = 0$ $k_A = 0$ $\rho_{AB} = 0$ $k_{AB} = 0$ $\theta_{.B} = 0$ $k_B = 0$ $k_{BA} = 0$
parallel	**III.** $\rho_A \neq 0$ $\rho_B \neq 0$ $\rho_{AB} \neq 0$ $\rho_{BA} \neq 0$ $k = 0$	**IV.** $k_A = 0$ $k_{AB} = 0$ $k_B = 0$ $k_{BA} = 0$

3. Application: modeling co-metabolism

Leadbetter and Foster [25] described the partial oxidation of certain hydrocarbons by *Pseudomonas methanica* growing on methane. These hydrocarbons did not support growth of the bacterium, but were 'co-oxidized'. Jensen [26] reported oxalate-utilizing strains of *Pseudomonas dehalogens* that liberated chloride from trichloroacetate, while they were unable to grow on this compound. Since 1959, many examples of similar phenomena have been reported. For instance, the transformation by methane mono-oxygenase of chlorinated compounds, like trichloroethane, has received considerable attention. In the literature, different names have been used to refer to the findings described above. Among them are co-oxidation [25], gratuitous or fortuitous metabolism [27], and co-metabolism [28]. In this paper, we use the term co-metabolism as it has become widely accepted.

The term co-metabolism has been defined to refer to transformations from which microorganisms do obtain neither energy nor 'nutritional benefit', cf. [29,30]. The current interpretation is less strict, as defined by Stirling and Dalton [27]: "*transformation of a compound, which is unable to support cell replication, in the requisite presence of another transformable compound.*" The former compound is referred to as co-metabolized or secondary substrate, whereas the latter is referred to as primary substrate. Further details about the term co-metabolism can be found in another article by Dalton and Stirling [31].

According to the current definition of co-metabolism, degradation of a secondary substrate may provide nutritional benefit, but the cell is unable to utilize it in absence of a primary substrate. The transformation of chlorinated aliphatics by methanotrophs, for example, fits well in this interpretation. For *Methylomicrobium album*, chloromethane cannot serve as sole growth substrate. But when co-fed with methanol, it enhances growth and its carbon is incorporated into the biomass for up to 38% [32]. Obviously, this example does not fit in the original 'no nutritional benefit' definition. Thus, the current view includes more substrates in the realm of co-metabolism. Furthermore, it seems to be more practical, since absence of benefits to the cell is not easy to confirm experimentally.

For different reasons, a primary substrate can be required to degrade a co-metabolite. First, co-metabolism can occur if the catabolic enzymes are not induced by the secondary substrate. This is exemplified by chloromethane that does not induce methane mono-oxygenase, and by 4-chlorophenol that does not induce

phenol oxidizing enzymes. Second, some co-metabolic transformations, as oxidations or reductive dehalogenations, require energy or reduction equivalents (e.g., NAD(P)H). Such transformations drain the cell's pool of reduction equivalents. However, the degradation of a primary substrate can make extensive catabolism of the secondary substrate possible by providing the necessary reduction equivalents. In this case, co-metabolism can reduce the biomass yield [33] and/or the maximum growth rate [33,34] on the primary substrate. However, the loss of reducing equivalents during co-metabolism does not always result in a (detectable) decrease in yield [34,35].

We can use the general framework above to model co-metabolism. Models described in literature [32–39] focus on the co-metabolism of structurally similar compounds. Here we show how our general framework can be used to model the co-metabolism of structurally dissimilar substrates. As can be seen from Fig. 3, we deal with a microorganism that carries out two transformations, $y_{AC}A \rightarrow C$ and $y_{BC}B \rightarrow C$. In terms of the conditions on the SU-parameters, substitutable-parallel implies $k_{BA} = k_B$, $k_{AB} = k_A$, and $k = 0$. Consequently, the expressions for the biodegradation rates (Eqs. (2) and (3)) reduce to:

$$j_A^+ = k_A(\theta_{A\cdot}^{ss} + \theta_{AB}^{ss}),$$
$$j_B^+ = k_B(\theta_{\cdot B}^{ss} + \theta_{AB}^{ss}). \quad (4)$$

In terms of the specific biodegradation rates, the production rate is given by $j_C = y_{CA}j_A^+ + y_{CB}j_B^+$. Substitutable-parallel degradation also implies $\rho_A \neq 0$, $\rho_{AB} \neq 0$, which means that substrate B does not inhibit the binding of substrate A. If in addition $\rho_B \neq 0$, $\rho_{BA} \neq 0$ holds, we deal with proper substitutable-parallel degradation (Fig. 1, diagram III). In contrast, if alternatively the conditions $\rho_{BA} \neq 0$ and $\rho_B = 0$ hold, microorganisms can only degrade B when A is also available. Substrate B is then a (xenobiotic) substrate that is co-metabolized with a (natural) substrate A as primary substrate. Under the conditions $\rho_A = \rho_{AB} \neq 0$, $\rho_{BA} \neq 0$, and $\rho_B = 0$, the expressions for the quasi-steady-state SU-fractions

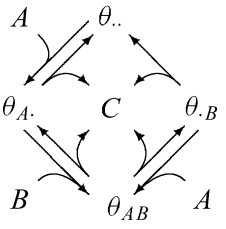

Fig. 3. Co-metabolism of a co-metabolite B and a primary substrate A. Notation as in Figs. 1 and 2. Substrate B can only bind to the SU–A complex and, consequently, the microorganisms can only degrade substrate B if substrate A is also available. Therefore, in contrast to the kinetics shown in Fig. 2, this scheme lacks an arrow pointing from $\theta_{..}$ to $\theta_{.B}$.

(θ^{ss}) become much more simple. Hence, the specific degradation rates (Eq. (4)) can be written as:

$$j_A^+ = k_A \frac{\rho_A j_A}{\rho_A j_A + k_A},$$
$$j_B^+ = k_B \frac{\rho_A j_A}{\rho_A j_A + k_A} \times \frac{\rho_{BA} j_B(\rho_A j_A + k_A + k_B)}{\rho_{BA} j_B(\rho_A j_A + k_B) + k_B(\rho_A j_A + k_A + k_B)}, \quad (5)$$

where j_B^+ is the specific biodegradation rate of the co-metabolized substrate. The production rate is given by $j_C = y_{CA}j_A^+ + y_{CB}j_B^+$.

Before going into the examples we rewrite Eq. (5) in terms of concentrations. In a well-mixed environment, the arrival rates of the compounds can be taken proportional to their concentrations. In mathematical terms this means $j_A = \alpha_A S_A$ and $j_B = \alpha_B S_B$. Substitution of these expressions into the equation for j_C above yields:

$$j_C = y_{CA}k_A \frac{S_A}{S_A + K_A} + y_{CB}k_B \frac{S_A}{S_A + K_A} \times \frac{S_B(S_A + K_A + (k_B/k_A)K_A)}{S_B(S_A + (k_B/k_A)K_A) + K_B(S_A + K_A + (k_B/k_A)K_A)}, \quad (6)$$

where the saturation constants are defined as $K_A = k_A/(\alpha_A \rho_A)$, $K_B = k_B/(\alpha_B \rho_{BA})$. The compound parameters $y_{CA}k_A$ and $y_{CB}k_B$ are the maximum production rates from substrates A and B, respectively.

According to the definition of co-metabolism above, biodegradation of the co-metabolite may result in the formation of new biomass. That is, the coefficient y_{CB} is not necessarily zero. Alternatively, the co-metabolite can exert a toxic effect on the microorganisms. This typically results in degradation rates that are low as long as the concentration of the toxic compound is high. The relation between co-metabolism and toxicity has been modeled in, for instance, [37–39]. For the moment, we do not take such toxic effects into account.

3.1. Experimental data analysis

In this section, we present two examples that illustrate how the model for co-metabolism can be applied in combination with the Monod model. The first example concerns the anaerobic growth of *E. coli* on citrate, whereas the second concerns the co-metabolic degradation of 3-chloroaniline.

To reduce the number of parameters of Eq. (6), we scale the substrate concentrations with respect to their saturation constants ($a = S_A/K_A$, $b = S_B/K_B$) and we write:

$$j_C = y_{CA}k_A \frac{a}{a+1} + y_{CB}k_B \frac{a}{a+1} \times \frac{b}{b + 1 - b/(a + 1 + k_B/k_A)}. \quad (7)$$

From this expression it can be seen how the handling rates influence the degradation process. Clearly, the values of a

and b are important in determining the amount of substrate B that is transformed. For high values of b, the amount of transformed B is proportional to the amount of transformed of A. The ratio of the handling rates (k_B/k_A) is also important. This ratio has more influence on the degradation rate of substrate B at low than at high scaled concentrations of substrate A (Fig. 4).

The model implements strict coupling between the co-metabolic degradation of substrate B and the uptake of the primary substrate A. This strict coupling between the consumption of growth substrate and co-metabolite has been reported for anaerobic growth of *E. coli* on citrate [7]. Citrate is almost completely degraded with glucose, lactose, or L-lactate as primary substrate. However, citrate breakdown stops when glucose is exhausted, whereas glucose breakdown continues after depletion of citrate [7]. To test if the new model is able to describe the co-metabolic consumption of citrate, we confronted it with an experiment by Lütgens and Gottschalk [7]. The following equations were used:

$$\frac{\mathrm{d}}{\mathrm{d}t}S_A = -k_A \frac{S_A}{S_A + K_A} S_C,$$

$$\frac{\mathrm{d}}{\mathrm{d}t}S_B = -k_B \frac{S_A}{S_A + K_A} \times \frac{S_B(S_A + K_A + (k_B/k_A)K_A)}{S_B(S_A + (k_B/k_A)K_A) + K_B(S_A + K_A + (k_B/k_A)K_A)} S_C,$$

$$\frac{\mathrm{d}}{\mathrm{d}t}S_C = y_{CA}\frac{\mathrm{d}}{\mathrm{d}t}S_A + y_{CB}\frac{\mathrm{d}}{\mathrm{d}t}S_B,$$

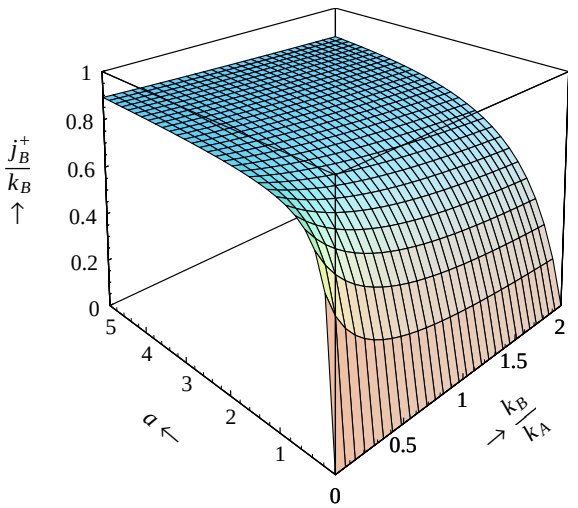

Fig. 4. The scaled degradation rate of substrate B (j_B^+/k_B) as a function of the ratio k_B/k_A and scaled concentration of substrate A ($a = S_A/K_A$, see Eq. (7)). The scaled rate has a maximum value of 1. The value of b ($= S_B/K_B$) is set to 10 for this simulation. Especially at low values of a, the ratio of the handling rates k_B/k_A influences the degradation rate of B. A larger value of this ratio, meaning that the handling rate of B increases with respect to that of A, requires a larger value of a to attain the same degradation rate.

where A refers to glucose, B to citrate, and C to biomass. The results are shown in Fig. 5. In the experiment, citrate was exhausted first. With the estimated parameter values, we carried out a model simulation in which glucose is exhausted first. The model indeed predicts that citrate consumption stops after the depletion of glucose (Fig. 5). Unfortunately, the authors [7] did not include the experimental data to support their statement.

The degradation of 3-chloroaniline (3CA) provides another example of co-metabolism [8]. In this case, the extent and rate of 3CA degradation depend on glucose concentration (Fig. 6), but 3CA also disappears from the medium when glucose is absent. This means that a 'background' degradation process is taking place, which is not related to the oxidation of primary substrate. However, as illustrated in the previous example, our model predicts co-metabolic degradation to stop once the primary substrate is exhausted. To describe the background degradation of 3CA, we extended the model to account for background degradation. We did so by adding to Eq. (6) the term $k_d S_B/(S_B + K_B)$, where k_d represents the maximum decay rate of 3CA in the absence of glucose. Fig. 6 shows the results of fitting this extended model against data from [8]. We conclude that the fit is quite acceptable.

4. Discussion

We introduced a model framework based on the innovative concept of synthesizing unit to model the four types of substrate interaction that can take place during the simultaneous utilization of two substrates. Although we described the framework with two substrates only, any number of substrates can be accommodated. To exemplify how this framework can be used, we developed a model for a specific type of co-metabolism, namely that of structurally non-analogous substrates.

Our framework, being a general and systematic model for multiple substrate utilization, cannot be directly applied to describe any situation that can occur during multiple substrate degradation. Sometimes the dynamics of intracellular pools may be extremely important and additional equations may then be needed, for example. Recently, Dahlen and Rittmann [40] formulated a model for multiple substrate degradation that, by means of a multiplicative Monod model, explicitly accounts for a NADH pool. Detailed information on the role of intracellular co-factors during substrate limitation and co-metabolism can also be found in [41,42]. If intracellular reserves and changes in the chemical composition of biomass cannot be ignored, application of the DEB theory, in which reserves are included [13] might be considered. Before it can be applied to describe certain systems, the general

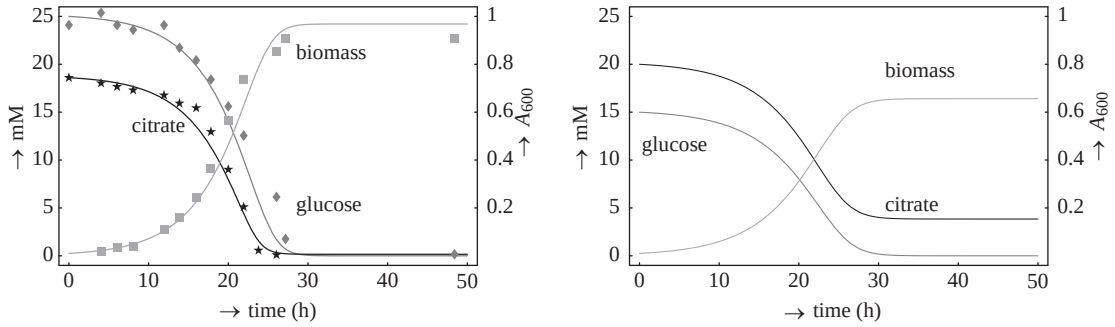

Fig. 5. Model fit and simulation of our co-metabolism model. Left: Model fit to data from [7]. *E. coli* consumes citrate anaerobically in the presence of glucose. Parameter values were obtained by fitting all data sets simultaneously. The Monod model was used to describe biomass growth. For the model equations, see text. Parameter values (A represents glucose, B citrate, and C biomass): $y_{CA} = 0.028^* A_{600}/\text{mM}$; $y_{CB} = 0.014\, A_{600}/\text{mM}$; $k_A = 140$ mM/(A_{600} day); $k_B = 132$ mM/(A_{600} day); $K_A^* = 6$ mM; $K_B^* = 2$ mM; $S_A(0) = 25$ mM; $S_B(0)^* = 18.6$ mM (measured value); $S_C(0)^* = 0.01\, A_{600}$ (initial value); $*$: parameter has been fixed during minimization. Right: Model simulation with the obtained parameter values with an initial concentration of glucose (S_A) of 15 mM and of citrate (S_B) of 20 mM. As can be seen from our simulation, glucose is consumed completely and citrate partly remains in the culture. Indeed, citrate consumption stops when the primary substrate (glucose) has been depleted as reported [7].

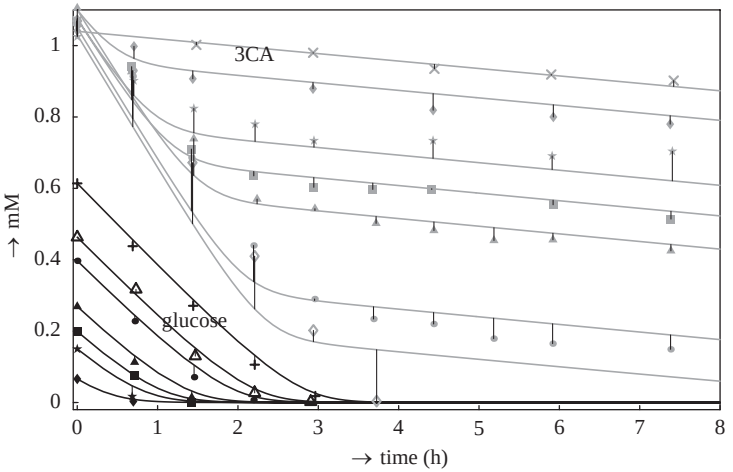

Fig. 6. Result of fitting our co-metabolism model to data from [8]. Co-metabolic degradation of 3-chloroaniline (3CA) by *Rhodococcus* with glucose as the primary substrate. The culture starting with 0.60 mM glucose serves as a control: 3CA is absent. The upper curves for 3CA (×) and for glucose (+) relate to the single substrate case. We fitted all 14 data sets simultaneously. The grey symbols and lines refer to 3CA data and fit, respectively. The black symbols and lines refer to glucose data and corresponding model predictions. Vertical lines indicate the difference between data points and model predictions. Parameter values (A represents glucose, B 3CA, and C biomass): $y_{CA}^* = 0.08$ mg dry weight/μmol glucose; $y_{CB}^* = 0$ mg dry weight/μmol 3CA; $k_A = 0.42$ mM/h/mg dry weight; $k_B = 0.60$ mM/h/mg dry weight; $K_A = 0.06$ mM; $K_B = 0.008$ mM; $k_d = 0.035$ mM/h/mg dry weight. The initial biomass concentration is 0.60 mg dry weight/ml. The initial glucose concentrations are 0, 0.07, 0.15, 0.20, 0.27, 0.40, 0.46, and 0.61 mM. The initial 3CA concentrations from top to bottom are: 1.04, 1.1, 1.075, 1.075, 1.1, 1.05, 1.03, and 0.0 mM. $*$: parameter has been fixed during minimization.

framework presented in this paper may need extensions to include phenomena like those mentioned above. These limitations are the necessary corollary of the advantages of a general model, however.

Let us remark four features that illustrate how our co-metabolism model relates to previous approaches. They are important to keep in mind when applying the model.

First, most modeling approaches have focused on co-metabolism of structurally analogous compounds. As competitive inhibition is often due to competition of structurally analogous substrates for the same binding

site, these approaches often assume that competitive inhibition takes place. In contrast, the general framework also allows us to describe co-metabolism in situations in which no competitive inhibition occurs. In Section 3.1, we view co-metabolism as a 'degenerate' *parallel*-substitutable interaction, whereas competitive inhibition is equivalent to *sequential*-substitutable interaction. Thus, our framework accounts for cross-competitive inhibition. With the examples (previous section) we showed that our model succeeds in describing the co-metabolic biodegradation of structurally non-analogous substrates. Please note that this concerns a specific type of co-metabolism.

Second, co-metabolism concerns the simultaneous metabolism of two compounds, where the degradation of a secondary substrate depends on the presence of a primary substrate. We incorporated this dependency into our model such that it involves the obligate presence of a primary substrate. Consequently, co-metabolic degradation only occurs if the primary substrate is present. However, degradation of the secondary substrate can continue after depletion of the primary substrate. In some situations, oxidation of dead biomass provides the necessary energy for background degradation, in other situations intracellular reserves provide it. The model can be extended to account for any of these situations, as shown in the previous section. If the rate of secondary substrate transformation is related to the rate of biomass decay or oxidation, biomass must be regarded as a substrate that provides energy.

Third, in our co-metabolism model the rate of secondary substrate transformation is a function of the concentration of both primary substrate and secondary substrate. Thus, as long as the concentration of secondary substrate is not toxic, its transformation rate increases with increasing concentration. This is in agreement with models that use cross-competitive inhibition to describe co-metabolism.

Fourth, we developed a general model for multiple nutrient utilization without using assumptions on intracellular pools of energy or reduction equivalents. Indeed, the general model connects biodegradation to substrate assimilation. This has the advantage that any microbial growth model can be used in conjunction with our model. This advantage also holds for our co-metabolism model being a special case of the general model.

As stated in Section 3, we did not take toxic effects into account in this paper. However, inhibition or deactivation of enzymes are important in, for example, co-metabolism of TCE by methanotrophs. Ely and co-workers [38] modeled the enzyme inactivation during TCE co-metabolism. Criddle and co-workers [43,44] developed comprehensive models for TCE co-metabolism that includes endogenous cell decay, product toxicity, and reducing power. Another important phenomenon, also occurring in TCE co-metabolism, is the induction of enzyme activity. A promising line of research could be to extend our model to accommodate such phenomena, which requires additional equations to describe their effect on synthesizing units. Since a separate module describes biomass growth, biomass decay is easily incorporated by adding a decay parameter to the growth module.

In summary, the general framework presented in this paper constitutes a useful tool for modeling several aspects of multiple nutrient utilization by microorganisms, such as the prediction of biodegradation rates and the analysis of multiple nutrient limitation. As an example, we showed how it can be applied to obtain a model that describes a particular type of co-metabolism, namely that of structurally non-analogous substrates. This model inherits the general model's flexibility and can, therefore, be combined with any microbial growth model, and can also be easily extended to account for background degradation, substrate loss due to physical processes, enzyme induction or adaptation, and product inhibition.

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