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Kinetics and interactions of BTEX compounds during degradation by a bacterial consortium

Jennifer V. Littlejohns, Andrew J. Daugulis *

Department of Chemical Engineering, Queen's University, Kingston, Ontario K7L 3N6, Canada

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ABSTRACT

A model to describe the biodegradation of benzene, toluene, ethylbenzene and *o*-xylene (BTEX) and growth of a bacterial consortium was systematically developed from a series of aerobic batch degradation experiments. The bacterial consortium was enriched from petroleum contaminated soil on a mixture of BTEX components and was identified to contain 7 unique species of *Pseudomonas*. Parameter estimates are reported for both conventional Monod parameters obtained from single substrate degradation experiments and interaction parameters obtained from dual substrate experiments. Key interactions identified include both the inhibition and enhancement of biodegradation rates for mixed substrates relative to single substrate experiments. Enhancement interactions have been qualitatively observed by other authors to occur to BTEX mixtures, but have not been quantified previous to the current study. Observations include the inhibition of benzene degradation in the presence of toluene and in the presence of ethylbenzene. As well, enhanced degradation of benzene was observed in the presence of *o*-xylene and toluene degradation was enhanced in the presence of benzene. A sum kinetics with interaction parameters (SKIP) model was found to accurately describe these interactions. In addition, it was found that *o*-xylene was cometabolized in the presence of toluene and/or benzene and a mathematical model was used to describe this interaction. The SKIP and cometabolism models were combined to predict both BTEX degradation as well as biomass production for this consortium when all BTEX components are present simultaneously.

1. Introduction

Benzene, toluene, ethylbenzene and *o*-xylene, collectively known as BTEX, are toxic compounds commonly emitted into the environment due to their ubiquitous presence in fuel and petroleum products. Biological processes are becoming increasingly popular for the elimination of these compounds from air [1], water [2] and soil environments [3] with the goal of achieving regulatory levels. Relative to thermochemical destruction methods, biological processes have inherent green benefits and potential cost savings. In addition, biological processes have the ability to effectively mineralize BTEX in low concentrations.

In order to properly design and model biodegradation processes it is necessary to determine the degradation kinetics of these compounds by bacterial communities. Degradation of combinations of BTEX components by pure bacterial strains has been studied, such as by bacterial cultures of *Rhodococcus rhodochrous* [4], several strains of *Pseudomonas putida* [5] and *Alcaligenes xylosoxidans* [6]. However, in order to efficiently degrade all BTEX components simultaneously a bacterial consortium is required, particularly for the removal of *o*-xylene, which has been found to be markedly persistent compared to other BTEX compounds [4,7].

The degradation of more than one growth limiting substrate by a bacterial population is not straightforward, as many different substrate interactions have been identified for combinations of BTEX components that can alter degradation rates relative to the absence of other substrates [4]. Such interactions can involve the enhancement or inhibition of degradation of substrates when in mixtures [8,9,10]. Inhibition of BTEX degradation by a bacterial consortium has previously been modeled using purely competitive inhibition kinetics [11], however enhancement interactions that have been qualitatively observed to occur during BTEX degradation by a consortium [8,12] have not been modeled to date. Moreover, an important stimulation interaction that has been observed to occur to *o*-xylene in the presence of other BTEX compounds is cometabolism [7] and models in order to describe the degradation of xylenes due to this interaction have been applied successfully [13].

* Corresponding author. Tel.: +1 613 533 2784; fax: +1 613 533 6637.
E-mail address: andrew.daugulis@chee.queensu.ca (A.J. Daugulis).

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Systematic approaches for determining substrate interactions during degradation by pure bacterial strains have been used for a mixture of three aromatic hydrocarbons [14] and three PAHs [15] and an identified and quantified bacterial consortia for a mixture of three aromatic hydrocarbons [16]. The present study successfully quantifies the kinetic parameters and substrate interactions for all four BTEX components during degradation by a bacterial consortium. As noted above, the only substrate interaction modeled for BTEX components to date has been competitive inhibition, and the current study serves to quantify additional interactions that have been observed only qualitatively. An appropriate model to describe interactions between all four BTEX components is systematically determined using dual substrate experimental data. The model structure is then verified by means of comparison to the experimental data for the degradation of all four components simultaneously. In addition, the current study investigates the use of the semi-empirical Monod model to predict biomass growth for a bacterial consortium with minimal knowledge of consortium composition. The developed model is shown to fit experimental data accurately.

2. Theory

For batch degradation biomass growth can be described by Eq. (1) [17], which can describe biomass growth due to a single or multiple substrates.

$$\frac{dX}{dt} = \mu X \tag{1}$$

Depletion of growth associated substrates in a batch degradation for a given substrate, i, can be described using Eq. (2).

$$\frac{dS_i}{dt} = -\frac{\mu_i X}{Y_{X/S_i}} \tag{2}$$

These equations hold true when maintenance requirements are negligible, which is typically assumed during the period of kinetic measurement of rapidly growing cells, as the metabolism of substrate is primarily growth associated [18]. It should be noted that the yield coefficient (Eq. (2)) must consider the consumption of compounds from both gas and liquid phases due to the volatility of BTEX, assuming that transfer between gas and liquid phases is rapid.

There are several models used to describe the specific growth rate for use in Eqs. (1) and (2). The most common model for the biodegradation of a single growth substrate, the Monod model, is shown as Eq. (3) [17].

$$\mu_i = \frac{\mu_{\max_i} S_i}{K_{S_i} + S_i} \tag{3}$$

Single substrate degradation experiments can be used to estimate the kinetic parameters $\mu_{\max}$ and K_s for each substrate. One method of estimating the kinetic parameters $\mu_{\max}$ and K_s involves fitting Eq. (3) to experimentally obtained specific growth rates as a function of substrate concentration for single substrate experiments.

Due to the toxic nature of BTEX and the possibility of substrate inhibition, a modified Monod model, the Andrews model, shown as Eq. (4) [19] may provide a better fit to experimental data obtained from single substrate experiments.

$$\mu_i = \frac{\mu_{\max_i} S_i}{K_{S_i} + S_i + S_i^2/K_I} \tag{4}$$

Again, experimentally obtained specific growth rates can be plotted as a function of substrate concentrations and fit to Eq. (4) to estimate the three kinetic parameters, $\mu_{\max}$, K_s and K_I.

The kinetic parameters, $\mu_{\max}$, K_s and possibly K_I, determined from single component degradation processes can be retained and used in specific growth rate models in which more than one growth limiting substrate is present. However, as stated previously, there is increased complexity in modeling multiple substrate degradation due to substrate interactions. Different models to describe the specific growth rate during the degradation of multiple interacting substrates have been developed in analogy to enzyme kinetics. The analogy can be made between enzyme kinetics and cellular kinetics because, if a reaction is enzyme catalyzed, then the inhibition of enzyme activity results in the inhibition of microbial growth by the same pattern [17]. The models used to account for these interactions can be used in substrate degradation equations (Eq. (2)). A common interaction for BTEX compounds is competitive inhibition, which can be seen in Eq. (5) [20]. During competitive inhibition, substrates compete for binding sites in order to be metabolized by the bacterial population.

$$\mu_i = \frac{\mu_{\max_i} S_i}{K_{S_i}(1 + (S_I/K_{S_I})) + S_i} \tag{5}$$

Another inhibition interaction is non-competitive inhibition wherein a nonreactive complex is formed when both substrates simultaneously are bound to one enzyme. This is shown as Eq. (6) [20].

$$\mu_i = \frac{\mu_{\max_i} S_i}{(K_{S_i} + S_i)(1 + (S_I/K_{S_I}))} \tag{6}$$

Uncompetitive inhibition is another interaction that can occur when multiple substrates are present, which is shown in Eq. (7) [20]. Uncompetitive inhibition is a situation in which one substrate can bind to only a substrate enzyme complex, not just the free enzyme.

$$\mu_i = \frac{\mu_{\max_i} S_i}{K_{S_i} + S_i(1 + (S_I/K_{S_I}))} \tag{7}$$

Finally, a model that accounts for substrate interactions without directly specifying the type of interaction is shown as Eq. (8) [21]. This model contains an interaction parameter that is treated as an unknown.

$$\mu_i = \frac{\mu_{\max_i} S_i}{K_{S_i} + S_i + I_{2_i} S_I} \tag{8}$$

Eq. (8) is called SKIP for sum kinetics with interaction parameters (SKIP) [14], which will become more evident as sum kinetics is described below.

In order to describe the growth rate of biomass when mixed growth substrates are present, sum kinetics, shown in Eq. (9) [21], can be used as an expression for specific growth rate, which can then be substituted into Eq. (1). Sum kinetics considers the contribution of each substrate present in a system to biomass growth.

$$\mu = \mu_1 + \mu_2 + \ldots + \mu_n = \frac{\mu_{\max_1} S_1}{K_{S_1} + S_1} + \frac{\mu_{\max_2} S_2}{K_{S_2} + S_2} + \ldots + \frac{\mu_{\max_n} S_n}{K_{S_n} + S_n} \tag{9}$$

The sum kinetics equation shown in Eq. (9) is for a situation in which there are no substrate interactions and simple Monod equations are summed. Furthermore, specific growth rate equations accounting for interactions, which have been described above, can be used in sum kinetics equations if interactions are found to occur among mixed substrates [11,14]. There is a limitation in using Eq. (9) substituted into Eq. (1) to describe biomass growth for a bacterial consortium, as a biomass formation equation should consider the biomass concentration able to