Aerobic cometabolic degradation of trichloroethene

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Summary and concluding remarks

8.1 Summary

Chlorinated aliphatic hydrocarbons (CAHs) form a group of environmental pollutants that can successfully be treated in bioremediation systems. Under aerobic conditions, a small number of these compounds, such as trichloromethane (chloroform) and the ubiquitous groundwater contaminant trichloroethene (TCE), can only be converted via cometabolism. In this process, the non-specificity of a cellular oxygenase enzyme, which is usually cofactor linked, is responsible for the 'accidental' transformation of the contaminant. Consequently, the natural (growth) substrate and the cometabolic substrate compete for the active site of the enzyme. Because the oxygenation products cannot be metabolized further, additional substrates are needed for growth and cofactor (NADH) regeneration. In addition, the intermediates formed during the chemical decomposition of the cometabolic conversion products may be reactive and harm the cell. Thus, in contrast to growth-based processes, cometabolic conversions are intrinsically unstable. The development of an aerobic cometabolic treatment system for the remediation of water contaminated with recalcitrant CAHs therefore constitutes a technological challenge. In this thesis, we focus on the development of an optimal bioremediation system for the compound TCE.

For application in an aerobic cometabolic bioremediation system the methanotroph *Methylosinus trichosporium* OB3b was, based on kinetic, economical, and environmental considerations, selected as the most suitable organism (Chapter 1). Since this organism is not ideal in all respects, some consideration is also given to the phenol-oxidizing organism *Burkholderia* (formerly *Pseudomonas*) *cepacia* G4 as an alternative.

To be able to accurately quantify the kinetics of growth and cometabolic TCE conversion of the organisms, a novel experimental technique was developed (Chapter 2). The technique is based on following the dynamic response to small perturbations of a chemostat culture by on-line measurement of the oxygen consumption rate. A mathematical model, incorporating microbial kinetics and mass transfer between gas and liquid phases, is used to interpret the data. The applicability of the technique is illustrated in the determination of 1) the affinity constants of strain OB3b under different conditions, and 2) the dynamic response of strain G4 to various substrate pulses.

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Since for strain OB3b, cofactor limitation resulting from TCE conversion appears to play an important role, a structured model was developed to evaluate strategies to supply the cells with NADH. In Chapter 3, the growth part of this model is presented. It is based on the metabolic pathways, includes Michaelis-Menten type enzyme kinetics, and uses NADH as integrating and controlling factor. An analysis of the physiological properties of the cells (flux control, enzyme levels, transient responses) is given to illustrate the applicability of the model.

In Chapter 4, the growth model is extended to include cometabolic TCE conversion, incorporating 1) the kinetics of contaminant conversion; 2) cofactor (NADH) use; 3) competitive inhibition between methane and TCE; and 4) toxic effects caused by the TCE conversion products. The model gives a realistic description of the experimental data and is used to optimize the process. A comparison of growth-based (single-step) and similar non-growth based (two-step) systems reveals that the highest TCE conversions per kg of cells grown are obtained in the latter. By adding small amounts of methane, NADH limitation in the second (TCE conversion) step can be eliminated. Thus, a very effective treatment system is obtained which makes optimal use of the TCE degradation capacity of the cells.

In Chapter 5, the same conclusion concerning the optimal set-up of the system is drawn, but now based on a qualitative analysis. Engineering implications are considered, and a simple unstructured model is presented to describe TCE conversion in the second step of the two-step process. The model, which assumes excess NADH availability, predicts very good performances with an optimal choice of the main design parameters, which are temperature, residence time, and inlet biomass concentration.

In two-step systems proposed thus far, large amounts of formate (20 mM) were used to prevent NADH limitation during cometabolic TCE conversion. Since in practice this may not be economically feasible, in Chapter 6 batch experiments are described aimed at reduction of the formate addition in a two-step system. Furthermore, experimental results from a small pilot-scale two-step set-up (50 l) are presented and compared to model predictions. The pilot-scale set-up appeared to perform very well. Optimization of the system (15°C) led to the conclusion that formate additions could be reduced by 75% (5 mM) without loss of TCE degradation capacity. Also, equally favourable results could be obtained by using methane instead of formate, implying a reduction in costs of chemicals of 85-90%. To describe the NADH limiting conditions (suboptimal formate and methane addition), the model presented in Chapter 5 had to be adapted. With parameters obtained from the batch experiments, the model appeared to give an accurate

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description of the overall TCE conversion measured in the two-step system.

The alternative strain considered, strain G4, is the subject of Chapter 7. Based on the results of the experimental work with strain G4, this organism can best be applied in a single step (growth-based) system, with phenol added at maintenance levels to preserve TCE degradation activity, and cell retention to achieve high volumetric TCE conversions and to prevent wash-out during shock loads. Comparing this optimized G4 system to the optimized two-step system of strain OB3b and systems proposed in literature, it could be concluded that systems using TOM-expressing cultures such as strain G4 and systems using methanotrophs such as strain OB3b, show similar good performances that are economically feasible compared to conventional treatment methods.

8.2 Concluding remarks

To date, no cultures have been characterized that grow on compounds like trichloromethane (chloroform) and TCE. Conversion of these compounds under anaerobic conditions is possible, but often leads to accumulation of less chlorinated, but sometimes more toxic, intermediates. During conversion under aerobic conditions via cometabolism no accumulation of toxic intermediates occurs, and this process is therefore preferable. Expectations based on the work described in this thesis, as well as a short recapitulation, serve to demonstrate practical implications.

For bioremediation systems based on cometabolism, two types of organisms appear to qualify, i.e. TOM-expressing cultures and methanotrophs.

Groundwater contamined with low concentrations (≈ 1 mg l^{-1}) of unsaturated chlorinated hydrocarbons (aliphatics and aromatics), can probably best be treated in a single-step biofilm system using a mixed culture of TOM-expressing organisms. Phenol should be added at maintenance levels, with adequate monitoring to ensure complete degradation. At these low contaminant concentrations, TOM-expressing cultures and methanotrophs have similar conversion rates, and the single-step systems for TOM-expressing cultures are preferred for simplicity. Landa et al.[124] has shown that the TOM-expressing strain G4 can recover from shock loads the system may occasionally be subjected to.

If higher concentrations need to be treated, such as may occur in industrial waste streams, or more complex matrices that also contain saturated organics, use of methanotrophic organisms is preferred. In that case, the best

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design involves two steps, with growth of a pure culture of strain OB3b separated from TCE conversion. Under these more demanding contaminant conditions, TOM-expressing cultures have lower conversion rates than methanotrophs and are too specific. Furthermore, due to the constant high contaminant loads, TOM-expressing cultures lack the time to recover from the effects of the toxic intermediates formed, and will eventually be totally inactivated. In the design of the methanotrophic systems, these toxic effects are accounted for, and this system is therefore preferable even though it is more complex and expensive than the single-step systems with TOM-expressing cultures.

Based on a cost evaluation carried out by Folsom et al.[78], it is expected that both strategies proposed can compete very well with the conventional methods involving air stripping and active carbon adsorption. For long term projects (>2 years), the biological solutions are expected to be economically more feasible. For the design of the cometabolism based bioreactor systems, the predictive models presented in the Chapters 6 (two-step) and 7 (single-step) are suitable.

Furthermore, the metabolic model described in the Chapters 3-4 may be used to increase the understanding of the cometabolic conversion process, as well as the metabolism of the cells in general. Thus, the model provides a useful tool for evaluating bioremediation strategies in which substrates are used with a complicated metabolic fate. Since it can easily be adapted to fit other applications, it may also be used to study, develop and optimize other processes in which NADH plays a crucial role.

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