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Two-Stage Dispersed-Growth Treatment of Halogenated Aliphatic Compounds by Cometalism

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■ A two-stage bioreactor that utilizes cometalic biotransformations for the treatment of halogenated aliphatics is proposed. Methanotrophic cells are grown in a dispersed-growth reactor prior to transferral to a plug flow transformation reactor in which they are contacted with the waste stream and transformation occurs. A model describing cometalic biotransformation is used together with basic equations for design of the growth and treatment reactors to predict treatment efficiencies and to evaluate the effects of the finite transformation capacity of resting cells, electron donor supply, and product toxicity on process design. For an example treatment scenario targeting trichloroethylene (TCE), methane transfer and growth reactor size are found to dominate the system design at high contaminant concentrations, while at low concentrations, the treatment reactor size becomes more important. The results of this analysis for a two-stage suspended-growth reactor system suggest that increasing methane and oxygen mass-transfer rates, cell yield, and transformation capacity may have a greater impact on reducing overall reactor size than would an increase in trichloroethylene transformation rate.

Introduction

The growing use of halogenated aliphatic compounds and their subsequent release into the environment indicates the need for the development of a low-cost, highly effective treatment system for their destruction. At present, the most prevalently used treatment processes for halogenated organics include air stripping and activated carbon sorption, which are capable of purifying water and gas streams, but simply transfer the organic contaminants

to a new phase without destroying them.

Many halogenated compounds such as trichloroethylene (TCE) have not been shown to be used by bacteria for energy or growth, but can be transformed through cometalism by organisms that use a primary substrate, such as methane, for metabolism (1-4). A treatment system based upon the cometalic transformation of halogenated aliphatics by methanotrophic microorganisms may be a cost-effective and efficient alternative to physical processes due to its potential for high transformation rates, complete compound degradation without formation of undesirable end products, applicability to a broad range of compounds, and a requirement for an inexpensive and widely available primary growth substrate.

Since methanotrophic TCE and methane oxidation both require the same key enzyme, competitive inhibition significantly affects the cometalic transformation kinetics, as evidenced both in suspended-growth (5) and unsaturated fixed-film bioreactors (6). Competitive inhibition must therefore be factored into process design. Previous studies with methanotrophic bioreactors have used single-stage reactors in which competitive inhibition makes optimization of transformation efficiency difficult (6-9).

However, methanotrophic cells are capable of transforming TCE in the absence of methane (resting cells), and in this way, competitive inhibition can be avoided. A recent finding of significance is that product toxicity and limited electron donor supply result in a finite transformation capacity (T_c) of resting cells (10). Here, T_c is defined as the maximum mass of TCE transformed by a unit mass of resting cells (mg of TCE/mg of cells); a corresponding term, the transformation yield (T_y), represents the maximum mass of TCE transformation per mass of CH_4 used to grow the cells (mg of TCE/mg of CH_4). Formate addition can significantly increase T_c and T_y , presumably due to the increased supply of electron

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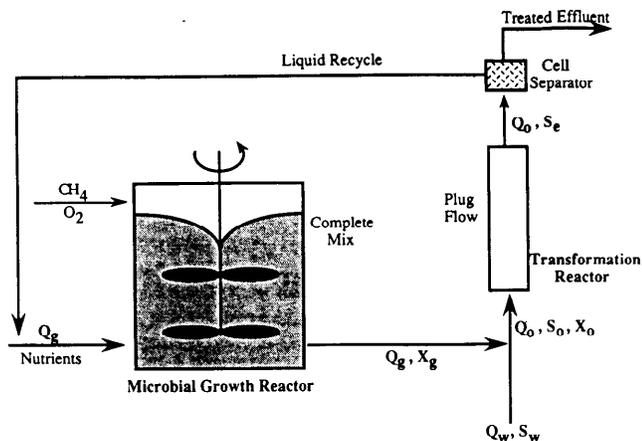


Figure 1. Proposed design for two-stage dispersed-growth methanotrophic treatment system for halogenated aliphatics.

donor produced during formate oxidation (11). Resting cell TCE transformation therefore is affected by the availability of an electron donor supply either from endogenous reserves or from an exogenous source such as formate. Formate addition to methanotrophic resting cells also results in an increased rate of TCE transformation (4, 12, 13) and hence may be useful in optimization of treatment efficiency. TCE transformation product toxicity, evidenced by greatly reduced methane and formate oxidation rates following TCE transformation (10, 14, 15), must also be factored into process design.

The finite transformation capacities of resting cells due to product toxicity or electron donor supply and competitive inhibition are phenomena that appear to be associated with cometabolic oxidation of many halogenated aliphatic compounds. The purpose of this paper is to incorporate these general concepts together with a cometabolic transformation model introduced previously (13, 16) into the design of a dispersed-growth cometabolic treatment system for chlorinated aliphatic compounds. A two-stage reactor is proposed that utilizes methane for cell growth in one reactor while conducting the transformation reaction in a second reactor (thereby excluding competitive inhibition). This work targets TCE for degradation since it is one of the most commonly encountered groundwater contaminants and is representative of a class of common solvents and their degradation products.

Proposed Cometabolic Treatment System

Figure 1 illustrates a two-stage treatment system designed to take advantage of the cometabolic transformation of halogenated aliphatic compounds, while recognizing the limitations imposed. The system consists of a growth reactor in which primary substrate is supplied to produce the organisms of interest under optimal conditions. The cell-rich growth reactor effluent is then mixed with the waste stream containing the compounds to be treated (contaminants), which together enter a treatment reactor without headspace where the contaminants are cometabolically degraded. When separate reactors for growth and contaminant transformation are maintained the reaction rate problems associated with competitive inhibition between the growth substrate and contaminant are also avoided.

The treatment reactor is designed in such a way that the transformation capacity of the cells is expended, or nearly so. Because of product toxicity, the cells are inactivated during treatment (14) and might be removed for disposal rather than being discharged or recycled back to the growth reactor. This is the purpose of the organism sep-

arator. Whatever water may be recycled back to the growth reactor is thus mainly devoid of organisms, but is functional for conveying the microorganisms between reactors. The design of the two reactors can be individually optimized by separating organism growth from treatment.

System Design Model

In the following, basic equations for design of the growth and treatment reactors are developed. The cell separator is not covered here, but there are many possible alternatives for this physical process.

Microbial Growth Reactor. The microbial growth reactor is considered to be a continuously stirred tank reactor (CSTR) without recycle. The reactor influent consists of a liquid stream containing inorganic nutrients and pH buffer for microbial growth. The primary substrate for energy and growth could also enter with the influent. However, with a methanotrophic reactor, the primary substrate is methane, which is preferentially transferred from a gas to the liquid phase. The relationship between hydraulic detention time, rate of methane utilization, and microbial cell concentration is as follows (17):

$$\frac{1}{\theta_g} = \frac{Q_g}{V_g} = \frac{Yr_m}{X_g} - b \quad (1)$$

The rate of primary substrate consumption is expressed as (15)

$$r_m = k_m X_g S_m / (K_m + S_m) \quad (2)$$

In addition, under steady-state operation of a methanotrophic bioreactor where growth is methane limited, the rate of methane consumption is equal to the rate of methane transfer from the gas to the liquid phase. Thus, an alternative expression is

$$r_m = k_L a \left(\frac{P_m}{H_m} - S_m \right) \quad (3)$$

By combination of eqs 1 and 3, the liquid volume of the microbial growth reactor is obtained as a function of the desired active microbial concentration X_g :

$$V_g = \frac{Q_g X_g}{Y k_L a \left(\frac{P_m}{H_m} - S_m \right) - b X_g} \quad (4)$$

Transformation Reactor. The rate of cometabolic transformation of contaminant by resting cells in the transformation reactor can be expressed as (13, 16)

$$r_s = k X S / (K_s + S) \quad (5)$$

The effective concentration of active microbial cells decreases as contaminant is consumed by cometabolism, as described previously (13, 16):

$$dX/dS = 1/T_c \quad (6)$$

Here, T_c is defined as the transformation capacity of the active cells. If the transformation reactor (Figure 1) were operated in a plug flow steady-state mode, then the contaminant concentration, S , would decrease with distance along the reactor. Likewise, eq 6 indicates that the active microbial concentration would decrease as well. The relationship between X and S at any point in the reactor is found by integration of eq 6 to give

$$X = X_0 - (1/T_c)(S_0 - S) \quad (7)$$

Substitution into eq 5 yields the transformation reaction rate as a function of S :

$$r_s = \frac{k \left[X_0 - \frac{1}{T_c} (S_0 - S) \right] S}{K_s + S} \quad (8)$$

From a mass balance on S at steady state, the relationship between the hydraulic detention time (θ_t) and contaminant transformation can be expressed as follows (18):

$$\theta_t = V_t/Q_0 = \int_{S_0}^{S_e} -dS/r_s \quad (9)$$

Integration of eq 9 yields the following relationship between detention time and effluent contaminant concentration for an idealized plug flow reactor:

$$\theta_t = \frac{-1}{k} \left[\left(\frac{K_s}{F_r X_0} \right) \ln \left(\frac{S_e/S_0}{\left(F_r + \frac{S_e}{T_c X_0} \right)} \right) + T_c \ln \left(F_r + \frac{S_e}{T_c X_0} \right) \right] \quad (10)$$

Here, a dimensionless term, F_r , defined as the residual capacity factor is introduced:

$$F_r = (X_0 - S_0/T_c)/X_0 \quad (11)$$

F_r represents the fraction of the transformation capacity that would remain if all the contaminant were completely consumed in the transformation reactor. If the cells are exposed to a concentration of contaminant greater than their overall transformation capacity (i.e., $S_0/T_c > X_0$), the resultant F_r will be negative and the contaminant will not be completely consumed. Alternately, when F_r is positive, the full transformation capacity of the cells will not be utilized under the given conditions and transformation of additional contaminant is possible.

For a continuously stirred tank reactor, a similar analysis leads to the following equation for steady-state operation:

$$\theta_t = \frac{1}{k} \left[\frac{(K_s + S_0)(S_0 - S_e)}{X_0 S_e \left(F_r + \frac{S_e}{T_c X_0} \right)} \right] \quad (12)$$

At the entrance to the transformation reactor, the waste flow (Q_w) is mixed with the growth reactor flow (Q_g), yielding the following as the influent microbial cell concentration (X_0) and contaminant concentration (S_0):

$$X_0 = X_g \frac{Q_g}{Q_w + Q_g} = X_g \left(\frac{R}{1 + R} \right) \quad (13)$$

$$S_0 = S_w \frac{Q_w}{Q_w + Q_g} = S_w \left(\frac{1}{1 + R} \right) \quad (14)$$

where R represents the flow ratio (Q_g/Q_w) and S_w the contaminant concentration in the influent waste stream. An implied assumption in the definition of S_0 is that the residual contaminant in the recycle stream (S_e) is destroyed in the growth reactor or otherwise lost. Assuming the transformation reactor is operated devoid of headspace, the transformation reactor volume, V_t , becomes

$$V_t = (1 + R)Q_w \theta_t \quad (15)$$

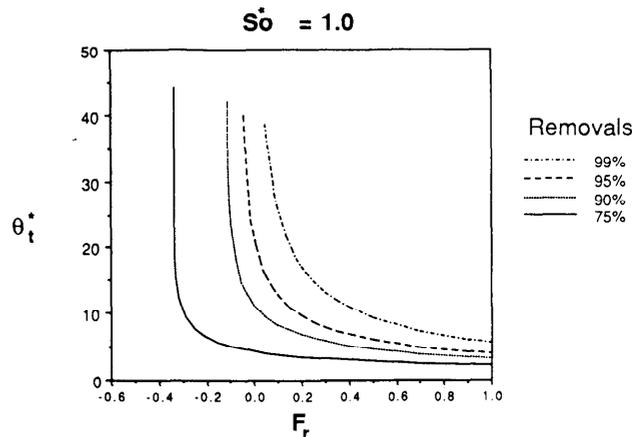


Figure 2. Relationship between dimensionless detention time (θ_t^*) and the residual capacity factor (F_r) for a dimensionless influent TCE concentration (S_0^*) of 1.0 over a range of removal efficiencies within a plug flow transformation reactor. F_r represents the fraction of cell activity remaining following the complete consumption of contaminant (TCE) within the transformation reactor.

System Characteristics

In order to illustrate the relationships between the different variables for reactor design, it is helpful to introduce dimensionless variables:

$$S_e^* = S_e/K_s \quad (16)$$

$$S_0^* = S_0/K_s \quad (17)$$

$$X_0^* = (T_c/S_0)X_0 \quad (18)$$

$$k^* = (t/T_c)k \quad (19)$$

$$\theta_t^* = (k/K_s)X_0 t \quad (20)$$

By introduction of these values into eq 10 for a plug flow reactor and rearrangement, the following equation is obtained:

$$\theta_t^* = \frac{1}{F_r} \left[\left[1 - S_0^* \left(\frac{F_r}{1 - F_r} \right) \right] \ln \left(F_r \left(1 - \frac{S_e^*}{S_0^*} \right) + \frac{S_e^*}{S_0^*} \right) - \ln \left(\frac{S_e^*}{S_0^*} \right) \right] \quad (21)$$

Here θ_t^* represents the dimensionless detention time and is a function of the dimensionless influent concentration, S_0^* , the fraction of contaminant remaining after treatment (S_e^*/S_0^*), and F_r . The residual capacity factor is already dimensionless and can be simplified to

$$F_r = \frac{X_0 - S_0/T_c}{X_0} = \frac{X_0^* - 1}{X_0^*} \quad (22)$$

Equations 21 and 22 indicate θ_t^* is a function of S_e^* , S_0^* , and X_0^* . It is of interest to examine how θ_t^* varies with F_r (and hence X_0^*). Figure 2 illustrates for $S_0^* = 1.0$ the relationship between θ_t^* , F_r , and a range of removal efficiencies expressed as a percentage [$100(S_0 - S_e)/S_0$]. Figure 3 illustrates the same relationship with 95% contaminant removal for a range of S_0^* values. For the case shown in Figure 2, 90% or better removal can be obtained at near-minimal detention times with F_r greater than zero, that is, when the transformation capacity of the cells does not become exhausted. Since θ_t^* tends to increase rapidly as F_r decreases below 0.2, a selected value of 0.2 or higher for F_r seems appropriate as a first estimate for treatment system design. Figure 3 indicates that with S_0^* of 1 or less (i.e., when $S_0 \leq K_s$), the values for θ_t^* and F_r show only slight dependence on S_0^* , but when S_0^* approaches 10 or

Removal = 95%

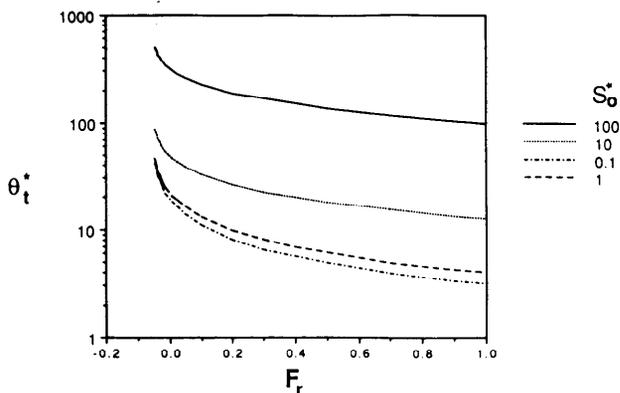


Figure 3. Relationship between dimensionless detention time (θ_t^*) and residual capacity factor (F_r) over a range of dimensionless influent TCE concentrations (S_0^*) for 95% removal in a plug flow transformation reactor.

Table I. Base-Case Coefficients Used in Growth and Transformation Reactor Design Examples

General Coefficients		TCE-Specific Coefficients	
$\theta_t = 9$ day	$Q_w = 10^3$ m ³ /day	TCE alone	TCE + formate
$K_{La} = 400$ day ⁻¹	$Y = 0.65$ g of cells/g of CH ₄	k , day ⁻¹	0.53
$H_m = 0.042$ atm·L/mg	$b = 0.1$ day ⁻¹	K_s , mg/L	0.37
$P_m = 0.09$ atm	$F_r = 0.2$	T_c , mg of TCE/mg of cells	0.036
$S_m = 0.02$ mg/L			0.080

higher ($S_0 \gg K_s$), θ_t^* increases in proportion to increases in S_0^* . This is a consequence of Monod kinetics: the reaction rate is first order with respect to substrate concentration at low concentrations, but zero order at high concentrations.

Design Examples

In order to illustrate the relationship between the respective volumes of the growth and treatment reactors, contaminated water characteristics, and treatment objectives, some examples for a completely mixed growth reactor with a plug flow treatment reactor are provided. Values used for the variables in the base case are listed in Table I. The general values listed in the first column represent operating conditions for the laboratory growth reactor previously described for the corresponding mixed-microbial population grown under nonaseptic conditions (10). Values for Y and b in the second column were determined specifically for the culture used (19), and the value of F_r is the minimum suggested in the preceding to limit treatment reactor size. The TCE-specific coefficients listed represent experimental results with the described mixed-microbial population and reflect the increased transformation rate and capacity of formate-fed cells (13).

Calculations of the system design factors are carried out as follows: X_g is computed from growth reactor coefficients by using eqs 1 and 3; the given F_r is used to compute X_0 (eq 11); R and Q_t are computed by using eq 13 with values of X_0 , X_g , and Q_w ; V_g is computed from eq 4, θ_t from eq 10, and V_t from eq 15. Table II is a summary of resulting system designs for treatment of contaminated streams with two significantly different concentrations of TCE in the waste stream (40 and 0.4 mg/L) in order to help illustrate the interplay between the growth and the treatment re-

Table II. Base-Case Example for Growth and Transformation Reactor Design Parameters Using Two Influent TCE Concentrations and a Removal Efficiency of 95%, $[100(S_w - S_e)/S_w]$, with Resting Cells Alone and in the Presence of Formate

	influent TCE conc (S_w) 40 mg/L		influent TCE conc (S_w) 0.4 mg/L	
	TCE alone	TCE + formate	TCE alone	TCE + formate
Growth Reactor				
cell density (X_g), mg/L	2610	2610	2610	2610
CH ₄ consumptn ^a , kg/day	3970	1790	40	18
volume (V_g , m ³)	4780	2150	48	22
Transformation Reactor				
% TCE removal	95	95	95	95
detention time (θ_t), day	0.096	0.030	0.492	1.37
volume (V_t), m ³	147	37	495	1370
Total System				
flow ratio (R)	0.53	0.24	0.0053	0.0024
net yield, ^b mg of TCE/mg of CH ₄	0.0096	0.021	0.0096	0.021
total volume ($V_g + V_t$), m ³	4930	2190	543	1400

^a Based upon measured cellular net yield of 0.35 mg of CH₄/mg of cells. ^b Represents the estimated mass of TCE consumed per unit mass of CH₄ consumed in reactors.

actors. With the higher influent contaminant concentration, the treatment reactor volume (V_t) is much smaller than for the low influent concentration, while for the growth reactor volume (V_g), the opposite is true. The sum total of reactor volumes is significantly lower for the low TCE concentration. At the high concentration, addition of formate to the treatment reactor to provide an exogenous energy source results in a significant reduction in size for both the treatment and growth reactors, whereas at the lower concentration, the treatment reactor size is surprisingly increased significantly by formate addition. The major reason for the latter is that at the lower concentration the transformation reaction rate is governed more by the ratio k/K_s than by k alone, and this ratio decreased when formate was added. This indicates that the interplay between variables is not always obvious and generalizations about the effect of system changes are sometimes difficult to make.

Table III contains the results of a sensitivity analysis for the base case without formate addition for the same two contaminant concentrations. The results of individually doubling the different important variables are illustrated. At high TCE concentration, the growth reactor size dominates the design. Two major design parameters are particularly significant here, the rate of mass transfer from the gas to the liquid phase (represented by variables k_{La} and P_m) and the transformation capacity (T_c). Increasing these variables can significantly reduce growth reactor size. While changes in other variables can reduce the transformation reactor size, this is not likely to lead to greatly reduced costs for high TCE concentration because of the dominance of the growth reactor size.

At low TCE concentrations, the treatment reactor comes into dominance, and efforts to reduce its size can significantly affect the overall costs. One of the most easily controlled variables here is F_r . When F_r is increased from 0.2 to 0.8, the treatment reactor volume is reduced from 495 to 60 m³. This effect is somewhat offset, however, by an increased methane consumption (from 40 to 159 kg/

Table III. Effect of Doubling Individual System Design Variables on Treatment System Characteristics for a TCE Removal Efficiency of 95 %

variable doubled	$R, Q_g/Q_w$	V_g, m^3	V_g, m^3	consumed $CH_4, kg/day$	net yield, ^c mg of TCE/mg of CH_4
		$S_w = 40 mg/L$			
base case ^a	0.53	147	4780	3970	0.0096
k	0.53	73	4780	3970	0.0096
K_s	0.53	154	4780	3970	0.0096
T_c	0.27	249	2390	1980	0.0192
$K_{L,a}$	0.27	125	2390	3970	0.0096
P_m	0.26	124	2380	3970	0.0096
$1/\theta_g$	0.81	169	3650	3970	0.0096
F_r^a	0.71	97	6370	5290	0.0072
F_r^b	2.12	42	19100	15900	0.0024
		$S_w = 0.4 mg/L$			
base case ^a	0.0053	495	48	40	0.0096
k	0.0053	247	48	40	0.0096
K_s	0.0053	892	48	40	0.0096
T_c	0.0027	986	24	20	0.0192
$K_{L,a}$	0.0027	493	24	40	0.0096
P_m	0.0026	493	24	40	0.0096
$1/\theta_g$	0.0081	496	37	40	0.0096
F_r^a	0.0071	263	64	53	0.0072
F_r^b	0.0212	60	191	159	0.0024

^a Base case makes use of variable values listed in Table I. ^b F_r value doubled again over value in row above to 0.8. ^c Represents the estimated mass of TCE consumed per unit mass of CH_4 consumed in reactors.

day) and an increase in the growth reactor size (from 48 to 191 m^3). A selection of the best value for F_r requires an economic evaluation, but a value of ~ 0.7 here provides the minimum total volume for growth and treatment reactors (224 m^3).

If eq 12 for a CSTR rather than eq 10 for the plug flow treatment reactor is assumed, then the treatment reactor size increases. For the case with 40 mg/L TCE and no formate addition, the CSTR treatment reactor size is 2–3 times larger than the plug flow case. For this concentration with formate addition, the CSTR is ~ 7 times larger, while at the lower contaminant concentration, with or without formate, it is ~ 10 times larger than the plug flow reactor. Thus, there can be considerable advantage in the design of a treatment reactor that approaches idealized plug flow.

The above examples illustrate that, with relatively high contaminant concentrations, methane transfer and the growth reactor size are likely to dominate the system capital and operating costs, while with low concentrations, the treatment reactor size would most likely dominate costs. The sizes of these reactors are affected by different system variables, and thus it is not clear which particular variables should receive most investigative effort for improvement. In order to successfully apply cometabolic treatment systems for biotransformation of halogenated aliphatic compounds, an understanding of the impact of all the system variables indicated is important.

Summary and Discussion

There have now been several reported studies on reactors for cometabolic transformation of TCE (6–9). The common operational characteristic of the suspended growth (7), unsaturated fixed-film (6, 8) and saturated fixed-film (9) systems reported to date is that growth and transformation have been conducted in the same reactor. When attempts have been made to increase methane transfer to the cells by increasing methane partial pressure in the gas phase, the contaminant transformation rate

often decreases because of competitive inhibition. Thus, with higher contaminant concentrations, where methane transfer has a dominant influence on reactor size, such reactors have significant limitations. In addition, possible toxic effects of contaminant or transformation products on microorganisms have not been well evaluated since the reactors were not operated sufficiently long under the steady-state conditions where toxic effects are likely to become evident.

The use of a two-stage biological treatment system for degradation of contaminants by cometabolism represents an attractive alternative when competitive inhibition between the primary substrate and contaminant is involved, and especially when the products of cometabolism are toxic to the microorganisms. This is the case with TCE transformation by methanotrophic bacteria (10, 14, 15), and also by organisms producing toluene dioxygenase (20, 21). It is probably true for any microbial oxygenase system that may be used for TCE transformation as the epoxide and its transformation products are known to be cytotoxic (22, 23). This is perhaps also true for other halogenated aliphatic compounds, although the degree to which substrate or product toxicity is exhibited in other cases is not yet well-known.

The two-stage reactor concept is related to the well-demonstrated phenomenon that resting methanotrophic cells grown under certain conditions, such as low copper concentration (3, 4), can transform halogenated aliphatic compounds at relatively high rates, comparable to the rates of transformation of the primary substrates themselves. The transformation yields (T_y) of resting cells tend to be of the same order of magnitude as commonly reported for actively growing cells, and thus separation into two stages appears not to result in a loss of efficiency in energy usage. It also provides the marked advantage of permitting cell growth to be optimized for high methane transfer rates and cell activity, using a growth medium that is best suited for this purpose.

This study has illustrated that there are two related characteristic values that affect the design of a two-stage cometabolic treatment system: the transformation capacity, T_c , and the transformation yield, T_y . These terms can be used to relate the energy requirement, in terms of quantity of primary substrate needed, to the amount of contaminant that can be degraded. The lower the value for T_y , the more primary substrate that is required to treat a given amount of contaminant. This generally translates into a larger required growth reactor because of limitations in gas transfer (oxygen in general, but methane also with methanotrophic systems). As illustrated in the examples provided, the growth reactor volume tends to dominate with higher substrate concentrations ($S_0 \gg K_s$), and thus for this case, increasing T_y results in a smaller growth reactor volume.

A high T_y corresponds to a high T_c , which is a limit set by the availability of internally stored reducing power, substrate toxicity, product toxicity, or some combination of all three. As illustrated with a methanotrophic culture (10, 13), T_c can be increased markedly by the addition of formate to the treatment reactor, which serves to increase the methanotrophic reducing power without resulting in competitive inhibition to TCE transformation or organism growth. Formate addition also increases the reaction rate markedly. However, as illustrated, this does not always translate into a smaller treatment reactor volume because K_s tends to increase markedly with formate addition as well. The advantage of added reducing power is effected mainly at high contaminant concentrations. There may

be other ways to increase T_c that need to be explored such as manipulating the cellular growth rate, optimizing the methane/oxygen ratio within the growth reactor, changing the microbial growth medium formulation, or reducing the toxic effects of the contaminant or its transformation products.

An interesting feature found from this reactor analysis is the confounding effects of the several variables on the size of the growth and treatment reactors, effects that tended not to be intuitively obvious. The use of F_r , the residual capacity factor, proved useful in this analysis. F_r indicates the fraction of T_c that will not be consumed during treatment. At higher contaminant concentrations ($S_0 \gg K_s$), designs with F_r as close to zero as practical tended to yield more optimal results because the growth reactor size was dominant. However, with low contaminant concentrations ($S_0 < K_s$), increased values of F_r appeared to provide more optimal reactor volumes as the treatment reactor size dominated here. Under these conditions, it becomes more important to take advantage of the maximum transformation rate by fresh cells, rather than maximizing the use of transformation capacity.

Because of the interplay between so many factors in the design of the two-stage system for cometabolic transformation of halogenated aliphatic compounds, an optimization model that includes capital and operating costs would be useful both for design and to guide future research on ways to reduce treatment costs. In addition, studies on cometabolism of other contaminants and their interactions when present in mixtures is desirable. However, present knowledge is sufficient to indicate that cometabolic treatment is technically feasible; the need is to increase economic feasibility.

Although much work has been done with methanotrophs to maximize the rates of transformation reactions with a range of nongrowth compounds (4, 10-12, 14, 15, 23), the results of the analysis presented here suggest that for a suspended-growth reactor system the development of methods for increasing methane and oxygen mass transfer, or increasing cell yield or transformation capacity, may have a much more significant impact.

Glossary

Growth Reactor

b	microbial decay rate (day^{-1})
H_m	Henry's law coefficient for methane ($\text{atm}\cdot\text{L}/\text{mg}$)
k_{1a}	gas/liquid mass-transfer coefficient (day^{-1})
K_m	primary substrate half-velocity constant (mg/L)
k_m	maximum rate of primary substrate consumption (mg of S_m (mg of cells) $^{-1}$ day^{-1})
P_m	partial pressure of methane in growth reactor headspace (atm)
Q_g	growth reactor influent and effluent flow rate (m^3/day)
r_m	rate of primary substrate consumption (mg/L^{-1} day^{-1})
S_m	steady-state primary substrate solution concentration (mg/L)
V_g	growth reactor volume (m^3)
X_g	steady-state active microbial concentration in growth reactor (mg/L)
Y	microbial growth yield (mg of cells/ mg of primary substrate)
θ_g	growth reactor hydraulic detention time (day)

Transformation Reactor

F_r	residual capacity factor
k	maximum rate of contaminant transformation (mg of S (mg of cells) $^{-1}$ day^{-1})
K_s	half-velocity constant for contaminant (mg/L)

Q_0	$Q_w + Q_g$, transformation reactor influent and effluent flow rate (m^3/day)
r_s	rate of contaminant transformation ($\text{mg L}^{-1} \text{day}^{-1}$)
S	aqueous contaminant concentration at time t (mg/L)
S_e	transformation reactor effluent contaminant concentration (mg/L)
S_0	transformation reactor influent contaminant concentration (mg/L)
T_c	transformation capacity of resting cells (mg of S/mg of cells).
T_y	transformation yield of resting cells (mg of S/mg of primary substrate).
V_t	transformation reactor volume (m^3)
X	active microbial concentration at time t (mg/L)
X_0	transformation reactor influent active microbial concentration (mg/L)
θ_t	transformation reactor hydraulic detention time (day)

General Terms

Q_w	waste stream flow rate (m^3/day)
S_w	waste stream contaminant concentration (mg/L)
R	Q_g/Q_w , overall reactor flow ratio

Registry No. TCE, 79-01-6; methane, 74-82-8.

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Survey of Potable Water Supplies for *Cryptosporidium* and *Giardia*

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■ The comparative occurrence of *Cryptosporidium* and *Giardia* was evaluated in 257 water samples from 17 states in the United States. *Cryptosporidium* oocysts were detected in 55% of the surface water samples at an average concentration of 43 oocysts/100 L, while *Giardia* cysts were found in 16% of the same samples at an average concentration of 3 cysts/100 L. *Giardia* and *Cryptosporidium* were more frequently detected in samples from waters receiving sewage and agricultural discharges as opposed to pristine waters. There was no correlation between the concentration of water quality indicator bacteria and either protozoa. Both protozoa were more frequently isolated in the fall than other seasons of the year. The concentrations of both organisms were significantly correlated in all waters. *Cryptosporidium* oocysts were detected in 17% of 36 drinking water samples (0.5-1.7 oocysts/100 L) while no *Giardia* cysts were detected. The widespread occurrence of cysts and oocysts in waters used as supplies of potable water suggests that there is a risk of waterborne transmission of *Cryptosporidium* and *Giardia* infections if the water is not adequately treated.

Introduction

Cryptosporidium and *Giardia* are enteric protozoa that cause waterborne disease. Waterborne giardiasis was first recognized in the United States in 1965, and as of 1988, 106 outbreaks have been reported (1). *Cryptosporidium* has only recently been recognized as a cause of waterborne disease. By the 1980s *Cryptosporidium* was well documented as a cause of diarrheal illness in humans and the first waterborne outbreak was reported in 1985 (2).

Giardia is currently the most frequently identified agent of waterborne disease in the United States; however, in the majority of outbreaks the etiological agent has remained undetermined (1). Although *Cryptosporidium* has been documented in only two waterborne outbreaks, it was responsible for one of the largest outbreaks in the United States since 1920, with an estimated 13 000 individuals affected (3). Not only was the size of the outbreak significant, but the water underwent complete treatment including coagulation, sedimentation, rapid sand filtration, and chlorination (4). Water quality standards for coliforms (<1/100 mL) and turbidity (<1 NTU) were met and disinfection (1.5 mg/L chlorine) was not deficient or inter-

rupted. However, improper or poor operational practices were identified, including poor mixing during coagulation and restarting of dirty filters without backwashing.

Many characteristics that enhance the potential for transmission through water are shared by *Cryptosporidium* and *Giardia*. Both are transmitted by the fecal-oral route, with the infected individual excreting *Cryptosporidium* oocysts or *Giardia* cysts. Animals as well as humans may serve as sources of environmental contamination and human infection. The oocyst and cyst are the environmentally stable stages and both are resistant to inactivation by drinking water disinfectants (5, 6). There is no simple or routine test that can be used to evaluate the occurrence of these protozoa in water, and the bacterial indicator system used to assess microbial water quality may be inadequate for the determination of parasitological water quality (7).

The occurrence of the enteric protozoa in drinking water sources indicates a potential risk for waterborne disease, depending on the level of contamination and the effectiveness of the drinking water treatment. In two previous surveys, 10 and 28% of the surface waters sampled were shown to contain *Giardia* cysts at levels between 0.6 and 5/100 L (8, 9). *Cryptosporidium* oocysts were reported in as many as 77% of the waters examined in the western United States at concentrations of 0.1-94 oocysts/100 L (10). In a study limited to a single watershed, *Cryptosporidium* oocyst concentrations in water were correlated to *Giardia* cyst levels (7).

This survey was undertaken to gain additional information on the comparative occurrence of *Cryptosporidium* and *Giardia* in waters used for potable supplies in the United States. In particular we were interested in the occurrence of cyst and oocyst levels in pristine (more protected watersheds) and polluted waters (receiving sewage and agricultural discharges), seasonal occurrence, and association with other water quality variables.

Materials and Methods

Samples were collected from rivers, streams, lakes (or reservoirs), and springs that were used as sources of drinking water. These sites were identified with the assistance of local water authorities and utilities. Samples were categorized as polluted on the basis of the description of the watershed including public access and use, development, farms, and known point discharges from sewage treatment plants, and as pristine if there was no or little human activity, restricted public access, no agricultural activity within the watershed, or sewage treatment plant

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