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Chapter 20

Bioprocessing of Environmentally Significant Gases and Vapors with Gas-Phase Bioreactors

Methane, Trichloroethylene, and Xylene

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Fixed thin film, gas/vapor phase bioreactors were assessed relative to their potential for the bioprocessing of methane, trichloroethylene (TCE), and *p*-xylene. Methanotrophic bacteria were used to process the methane and TCE while a xylene resistant strain of *Pseudomonas putida* was used to process the *p*-xylene. Comparisons between the gas phase bioreactors and conventional shaken cultures and sparged liquid bioreactors showed that the gas phase bioreactors offer advantages over the other two systems for the degradation of methane in air. Rates of methane removal with the gas phase bioreactors were 2.1 and 1.6 fold greater than those exhibited by the shaken cultures and sparged liquid bioreactors, respectively. The gas phase bioreactors were shown to have application for the removal of TCE vapors from air with a removal rate of approximately 9 μg TCE d^{-1} $bioreactor^{-1}$. Xylene vapors were also scrubbed from air using gas phase bioreactors. At a feed rate of 140 μg of xylene min^{-1}, approximately 46% of the xylene was mineralized to carbon dioxide in a single pass through a bench scale gas phase bioreactor.

The biological processing of environmentally significant gases and vapors such as volatile halocarbons and hydrocarbons is becoming a topic of ever-increasing importance. Unacceptably slow degradation rates are a significant problem in the bioprocessing of these types of compounds due, at least in part, to the gas/vapor-in-water solubility limitations inherent in conventional aerated liquid phase bioreactors. Fixed thin film, gas/vapor phase bioreactors designed specifically for the bioprocessing of gases and vapors offer a potential means to partially combat this gas-in-liquid solubility problem. This chapter discusses results obtained with

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prototype laboratory scale gas/vapor phase bioreactors used for bioprocessing three different gases/vapors: methane, trichloroethylene (TCE) and xylene. The methane and TCE were bioprocessed with methanotrophic bacteria, and the xylene with a xylene metabolizing strain of *Pseudomonas putida*.

Background. Methanotrophic bacteria have been known and studied for the past 85 years (*1*). During this period, the basic physiological capabilities of these organisms were elucidated with their ability to sequentially oxidize methane in the presence of air (O_2) to carbon dioxide and water being particularly well defined (*2*).

In recent years increased emphasis has been placed on exploiting the physiological potential of the methanotrophs. Areas of interest include bioconversion of methane to alternate and potentially valuable products such as single cell protein, methyl ketones, etc. (*3*), control of methane emissions resulting from coal mining *(4)* (Apel, W. A.; Dugan, P. R.; Wiebe, M. R; "Influence of Kaolin on Methane Oxidation by *Methylomonas methanica* in Gas Phase Bioreactors" Fuel, in press), and degradation of environmentally significant low molecular weight halocarbons like trichloroethylene (*5-9*). As a result of these interests, development of bioreactor systems allowing more efficient methanotrophic conversion of gases and vapors are of considerable relevance.

Traditionally, production of the large amounts of methanotrophic bacteria required for the above applications has been accomplished by growing the organisms on methane/air mixtures added to liquid cultures (*10,11*). An inherent limitation of this method is the relatively limited solubility of methane and air in the liquid phase so that these gases are not readily available to the bacteria in the quantities necessary to sustain maximum reaction rates. Various techniques have been employed to combat this problem including mechanical agitation and sparging of methane/oxygen or methane/air mixtures through the cultures in an attempt to continuously saturate the liquid culture medium with the necessary gases.

One approach to increasing gas delivery to the methanotrophic bacteria is culturing the organisms as a thin biofilm on inert supports suspended in a gas or vapor phase. In such a system, gas and/or vapor availability to the cells can be increased. As such, it should be theoretically possible to increase methanotrophic gas and vapor removal rates by making the necessary gases or vapors more readily available to the organisms.

While this approach is particularly well suited to methanotrophs due to their use of a gas (i.e. methane) as a carbon and energy source, the same approach should be applicable to other bacteria which are capable of bioconverting other gases and vapors. The biodegradation of volatile, higher molecular weight organic compounds such as those in the BTEX group (benzene, toluene, ethylbenzene and xylene) are of particular interest due to their association with hydrocarbon fuel spills which are often, at least in part, remediated biologically. Certain Gram (-) heterotrophic bacteria are useful in the conversion of BTEX contaminants since they have been shown to be resistant to high concentrations of compounds like xylene and capable of metabolizing these compounds as their sole carbon and energy source (*12*). The catabolic processes by which these types of organisms

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process aromatic hydrocarbons vary but usually involve the oxidation of the aromatic substrate to a diol such as catechol followed by cleavage to a diacid such as *cis, cis* muconic acid (*13*). The diacid is then further metabolized, ultimately to carbon dioxide and water.

This paper reports the results from a series of experiments utilizing fixed, thin film, gas phase bioreactors for (a) culturing methanotrophic bacteria and comparing rates of methanotrophic methane removal in gas phase bioreactors vs. conventional shaken and sparged liquid cultures, (b) evaluating the feasibility of using the methanotrophic gas phase bioreactors for TCE degradation, and (c) the degradation of xylene by *Pseudomonas putida*.

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Materials and Methods

Culture Techniques. The *Methylomonas methanica* 739 used in these experiments was provided by the Ohio State University Department of Microbiology (Columbus, OH, U.S.A.) while the *Methylosinus trichosporium* OB3b isolate used was from the Oak Ridge National Laboratory culture collection (Oak Ridge, TN, U.S.A). Both cultures were maintained in CM salts medium as previously described (*4*).

The strain of *Pseudomonas putida* employed for the xylene degradation studies was isolated by two of the authors (J.H.W. and R.D.R.) as previously reported (*12*). Cultures were grown on a mineral salts medium consisting of the following components per liter: 0.7g KH_2PO_4, 0.5g $(NH_4)_2SO_4$, 0.3g $MgSO_4$ with traces of $FeSO_4 \bullet 7H_2O$, $MnCl_2 \bullet H_2O$ and $NaMoO_4 \bullet 7H_2O$. This mineral salts medium was aseptically dispensed in 50 ml aliquots into sterile 125 ml serum bottles which were sealed with teflon coated rubber septa. The bottles were gassed with air which had been saturated with xylene. Xylene was the organism's sole carbon and energy source. Incubations were performed at 22±2°C on a gyratory shaker at 200 rpm. Cultures were transferred at least biweekly to maintain viability.

Liquid Culture Studies. Liquid culture methane depletion studies were performed using *M. methanica* at stationary phase in 50 ml of CM salts contained in 125 ml serum bottles sealed with teflon coated septa. The bottles were gassed with various concentrations of methane-in-air ranging from 5 to 40% (v/v) and incubated in inverted position at 22±2°C on a gyratory shaker set at 120 rpm. Head space gas samples were periodically removed and analyzed for methane, oxygen and carbon dioxide as described below.

In a similar manner, methane depletion studies were also performed in liquid cultures contained in columns exactly like those used for the methanotrophic gas phase bioreactor studies (see description below) except that the Pall Rings were eliminated from the column and the column was filled with CM salts medium to a volume equal to that displaced by the Pall Rings in the gas phase bioreactor studies. Methane depletion rates were determined with a variety of methane/air mixtures ranging from 2 to 46% (v/v). In these studies, the methane-in-air mixtures were recirculated in an up flow direction through the column at 200 ml min^{-1}. The bioreactor was incubated at 22±2°C. Head space gas samples from

the liquid bioreactor were periodically analyzed for methane, oxygen and carbon dioxide as described below. These experiments were performed to give a direct comparison of methane removal rates by methanotrophic bacteria in similarly configured sparged liquid and gas phase bioreactors.

Gas Phase Bioreactors. The gas phase bioreactors used for experiments with methanotrophic bacteria were constructed by filling a 7.62 X 76.2 cm glass column with 1.6 cm (5/8 in) Pall Rings (Norton Chemical Process Products Division, Akron, Ohio) and sealing the open end with a rubber stopper (Fig. 1). The Pall Rings served as inert supports for the growth of thin layers of biofilm composed of the methanotrophic bacteria. The seal was further secured by over wrapping the boundary area between the stopper and the column with parafilm. A closed loop for gas recirculation through the bioreactor was constructed using flexible 0.4 cm o.d. teflon tubing connected to the upper and lower ends of the bioreactor. Included in the loop was a 1 l Erlenmeyer flask to increase the gas volume of the system and a peristaltic pump to recirculate the gas. The gas was circulated in an up flow direction through the bioreactor at a rate of 200 ml min^{-1}. Approximately 100 ml of CM salts medium was maintained in the base of the bioreactor to humidify the recirculating gas mixture. Total system volume was measured by water displacement and found to be 4.5 l. It should be noted that unlike the xylene studies reported below, all methanotrophic experiments were conducted as closed loop, batch bioreactor studies.

The gas phase bioreactors to be used in methane depletion experiments were inoculated with *M. methanica* and incubated as previously described (*4*) until the bioreactors reached steady state for methane-oxygen uptake and carbon dioxide evolution. This occurred approximately 6 weeks after inoculation at a methane uptake rate of 40 mg methane hr^{-1} when feeding the bioreactors a 30% methane-in-air gas mixture. This rate was maintained for several months by draining the humidification heel of CM salts medium from the base of the bioreactors and pouring 100 ml of fresh, sterile, CM salts medium over the supports at approximately 1 month intervals. The fresh CM salts solution was allowed to collect as a new humidification heel in the bottom of the reactor. Methanotrophic bacteria grew in this heel, but studies demonstrated no detectable methane uptake could be attributed to these organisms vs. those growing on the supports in the gas phase. With the exception the volume occupied by this heel, the void volume of the reactor was maintained in the gas phase. All methane uptake rate studies were performed in the steady-state gas phase bioreactors at 22 $\pm$ 2°C. For methane depletion studies both the liquid cultures and the gas phase bioreactors were charged with various concentrations of methane in air. Methane, oxygen, and carbon dioxide levels were monitored in the gas phase bioreactors using the methodology described below. Rates of methane uptake per unit of biomass per unit time were calculated from these data.

Gas phase bioreactors used for TCE degradation studies were prepared as indicated above except they were inoculated with *M. trichosporium* instead of *M. methanica*. This change in microorganism was necessary since previous screening studies had shown essentially no TCE degradation by *M. methanica* 739

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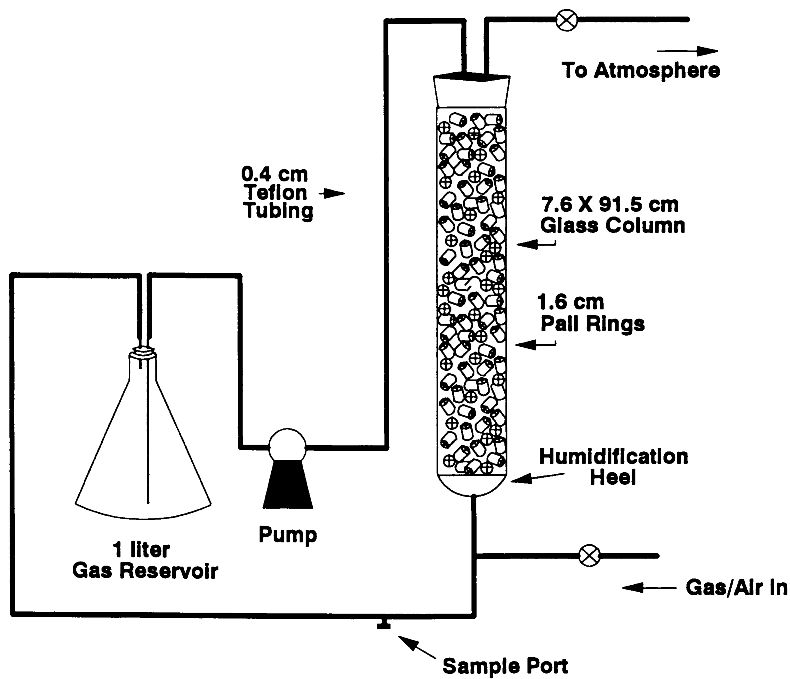

Figure 1. Schematic diagram of gas phase bioreactor configured for bioprocessing with methanotrophic bacteria.

and excellent rates of TCE degradation by *M. trichosporium* OB3b. Copper was deleted from the CM salts medium used. Researchers have shown that the presence of copper inhibits the expression of soluble methane monooxygenase, the enzyme believed to be responsible for TCE degradation by *M. trichosporium* (*14*). For each experiment, the bioreactor was flooded with 5% methane in air (v/v) with TCE added as a vapor to the recirculating gas stream. A second uninoculated bioreactor was run in parallel to the inoculated bioreactor to serve as an abiotic control to account for TCE loss through adsorption, etc. Abiotic control bioreactors used in all experiments described in this paper consisted of bioreactors structurely identical to the inoculated bioreactors. The abiotic bioreactors were steam steriled by autoclaving for 30 min. at 240 p.s.i. and all gas and liquids introduced into the abiotic control were filter steriled directly prior to introduction. Gas samples were removed from the bioreactors, and TCE, methane, oxygen, and carbon dioxide concentrations were analyzed as described below.

The gas phase bioreactors for xylene degradation by *Pseudomonas putida* were constructed in a manner similar to that described above except that the bioreactors were configured for a single pass of the gas/vapor phase (i.e. as a plug flow reactor) and a counter current basal salts medium feed was established (Figure 2). Specifically, the bioreactor consisted of a 7.6 X 91.5 cm glass process pipe column with 5.1 X 7.6 cm glass reducers fitted to each end via stainless steel clamps with teflon seals. Custom machined, teflon plugs were attached to the small ends of each reducer using standard process pipe stainless steel clamps with teflon seals. These plugs were drilled and tapped to accept tubing adapters. Both gases and liquids were transported through 0.4 cm o.d. teflon tubing. The column was filled with 1.6 cm (5/8 in) polypropylene Pall Rings. The column was sterilized and inoculated with *P. putida* by slowly dripping a pregrown stationary phase culture of *P. putida* over the supports for a period of approximately one week. A xylene/air mixture (approximately 2000 μg *p*-xylene min^{-1} at a gas/vapor flow of 55 ml min^{-1}) was then fed in an up-flow direction through the bioreactor for an additional two weeks. The entire void volume of the bioreactor was maintained in a gas phase. During experimental operation, the column was fed with a gas stream consisting of air that had been sparged through a *p*-xylene reservoir. The *p*-xylene carried by the air stream was the sole carbon source entering the bioreactor. Gas samples were removed from sampling ports as noted in Figure 2. These samples were analyzed for xylene, carbon dioxide and oxygen concentrations as described below. In addition, samples were removed from the liquid effluent stream exiting the bioreactor and analyzed for xylene, total inorganic carbon (TIC), and total organic carbon (TOC) as described below. The flow rates of the gas stream entering and exiting the bioreactor as well as the flow rate of the liquid effluent stream were determined at the time samples were withdrawn for chemical analyses. The flow rates together with the results from the chemical analyses were used to calculate carbon mass balances through the bioreactor.

Biomass development in the gas phase bioreactors was periodically monitored by weighing the gas phase bioreactors and comparing changes in weight to an original tare weight established for each of the bioreactors immediately after assembly. The weight of each bioreactor was then subtracted from the original

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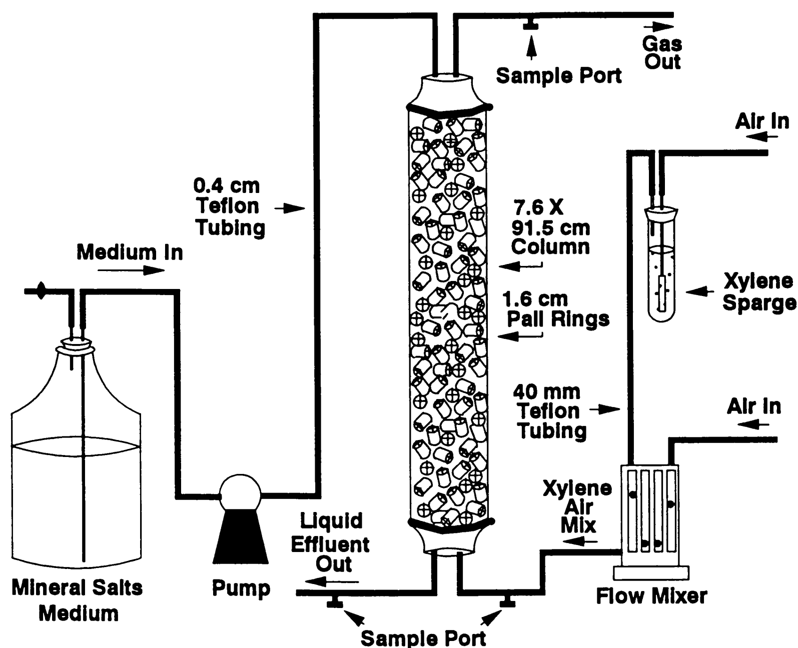

Figure 2. Schematic diagram of gas phase bioreactor configured for single pass bioprocessing of xylene vapors.

tare weight to determine a wet weight value for biomass in the bioreactor. Several differnt samplings of wet biomass were withdrawn from the bioreactors and dried to establish a conversion factor between wet weight biomass and dry weight biomass. This conversion factor was then used to calculate dry biomass weights from the wet weight measurements.

Analytical Methods. Gas levels (methane, oxygen, and carbon dioxide) in the serum bottle cultures and the bioreactors were analyzed using a Gow-Mac Series 550P gas chromatograph equipped with a thermal conductivity detector and an Alltech CTRI column. The gas chromatograph was connected to a Hewlett Packard model 3390A integrator. Samples consisted of 600 μl gas volumes manually injected with a gas tight syringe into the gas chromatograph. Helium was the carrier gas at a flow rate of 60 ml per minute. The gas chromatograph was operated under isocratic conditions at 30° C.

TCE was measured by removing 100 μl samples from the bioreactor which were analyzed using a Hewlett Packard 5890A gas chromatograph (Avondale, PA) equipped with an electron capture detector and an Alltech 624 non-packed column. The detector temperature was 260°C and the injection temperature was 225°C. The carrier gas was helium at 5 ml min^{-1} and the auxiliary gas was nitrogen at 65 ml min^{-1}.

Xylene vapors were analyzed by removing samples from the gas inlet sampling port and the gas outlet sampling port of the bioreactor using a 100 μl gas tight syringe. The xylene was quantified by injecting the 100 μl sample into a Sigma 4B Perkin-Elmer gas chromatograph (Norwalk, CT) equipped with a flame ionization detector and a Supelco SP-1500 Carbopak B column. The carrier gas was helium at a flow rate of 33 ml min^{-1}. The oven temperature was 220°C. The air flow rates through the bioreactor were determined at the sampling points using a bubble flow meter. The flow rates together with the results from the chromatographic analyses were used to calculate flow per unit time of xylene contained in the gas streams entering and exiting the bioreactor.

Xylene was analyzed in the liquid effluent by taking samples from the effluent stream sampling port at the base of the bioreactor. The samples were placed in 2 ml serum vials containing 0.05 ml of 2% $HgCl_2$. The vials were then sealed with a teflon lined septum. These samples were analyzed using the same method as that described for the xylene samples in the vapor phase except a 1 ul sample was injected into the gas chromatograph. The liquid flow rate of the effluent from the column was measured at the time of sampling and used in conjunction with the gas chromatography analyses to calculate the flow per unit time of xylene in the liquid effluent from the bioreactor.

The liquid effluent samples were also analyzed for carbon dioxide and other forms of carbon on a Model 700 TIC/OC Analyzer (O-I Corporation, College Town, TX). A 500 μl sample was introduced by syringe injection. A 200 μl volume of a 5% (v/v) solution of phosphoric acid and 500 μl of a 100 g l^{-1} solution of sodium persulfate (Fisher Scientific) were added to each sample. Potassium biphthalate (Fisher Scientific) was used as the organic standard.

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Results and Discussion

Methane removal data over a range of methane concentrations by *M. methanica* in the shaken serum bottles, the liquid bioreactor and the gas phase bioreactor are represented in Figures 3-5, respectively. All methane removal experiments were conducted with the methanotrophic cultures in stationary growth phase, and the data are reported as methane contained in the gas phase of the bottles or the bioreactors at the time in question. The data in Figures 3-5 are on a per bottle or bioreactor basis and represent gross methane removal unadjusted for biomass densities.

The data contained in Figures 3-5 are summarized in Figure 6 in terms of methane removal rates per unit time per g dry wt of biomass over a number of initial methane concentrations ranging from 1% to 53% methane-in-air for each of the three test systems. The shaken serum bottles exhibited relatively constant methane removal rates of approximately 3.8 mg methane hr^{-1} (g dry wt of biomass)$^{-1}$ at initial concentrations of methane-in-air up to approximately 40%. Above 40%, methane removal rates appeared to decrease as initial methane concentration increased.

In general, methane removal rates by *M. methanica* in the sparged liquid bioreactor increased as a function of increasing methane concentration in air to a maximum removal rate of 5 mg methane hr^{-1} (g dry wt biomass)$^{-1}$. This maximum occurred at a 22.5% initial methane-in-air concentration. At higher methane-in-air concentrations the removal rates appeared to decrease as initial methane concentrations increased.

The results obtained with the gas phase bioreactor tended to parallel those noted with the sparged liquid bioreactor. Methane removal rates increased as a function of increasing initial methane concentrations up to a 28% methane-in-air concentration. At this point the methane removal rate was approximately 8 mg methane hr^{-1} (g dry wt biomass)$^{-1}$.

The observed increase in methane removal rates as a function of increasing initial methane concentration was anticipated, and was almost certainly the result of two interrelated factors: (1) the kinetics of the methanotrophic enzyme system, and (2) transport of the substrate across the phase boundary and diffusion through the biofilm to the methanotrophic cells. Until the methanotrophic enzyme system is saturated with substrate, in this case methane, the system would be expected to follow first-order reaction kinetics with rates of reaction increasing as substrate concentration increases. At levels equal to or greater than enzyme saturation, the degradation kinetics would be expected to become zero-order with the degradation rate becoming independent of substrate concentration. Likewise, methane concentration in the gas phase influences the physical availability of methane to the cells in the biofilm. At gas phase levels below those sufficient to saturate the biofilm, transport of the methane across the boundary layer and further diffusion of the methane through the biofilm to the methanotrophic cells could be the rate limiting step. At gas phase methane concentrations equal to, or greater than the biofilm saturation point (i.e. the critical concentration), the biofilm would be fully

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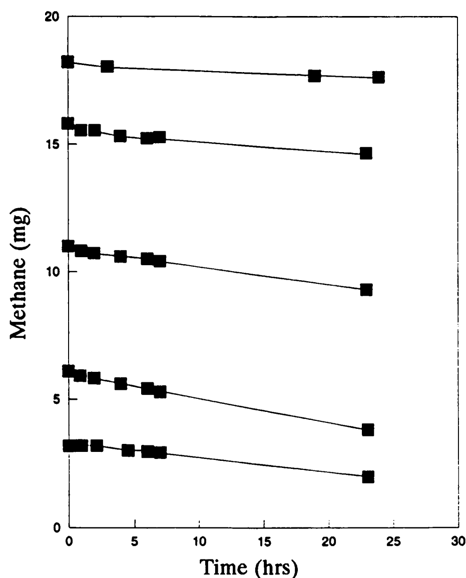

Figure 3. Removal of methane at various initial concentrations by *Methylomonas methanica* grown in serum bottles at 22±2° C. (125 ml serum bottles containing 50 ml medium, methane measured as gas in head space)

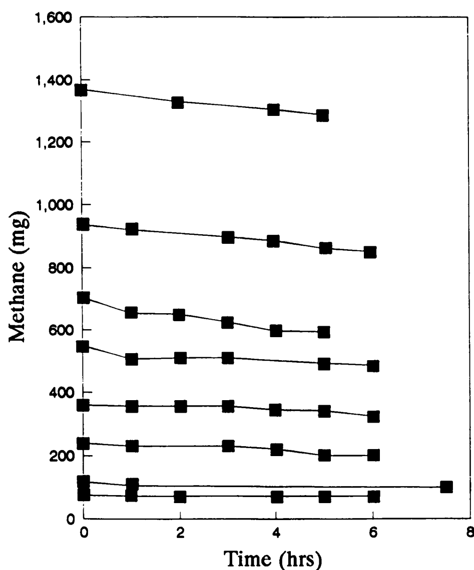

Figure 4. Removal of methane at various initial concentrations by *Methylomonas methanica* grown in liquid phase bioreactors at 22±2° C.

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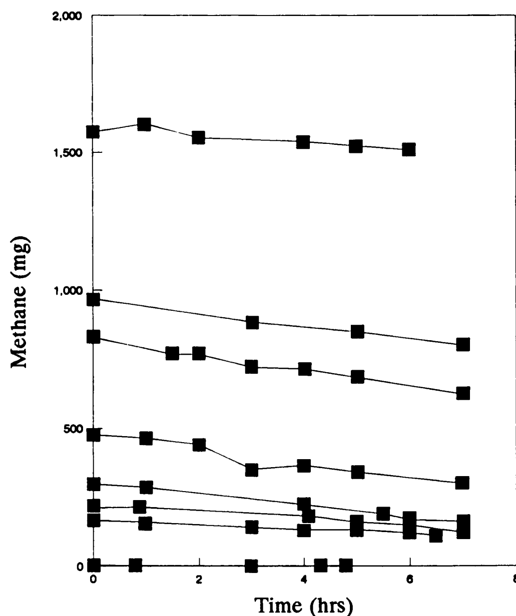

Figure 5. Removal of methane at various initial concentrations by *Methylomonas methanica* grown in gas phase bioreactors at 22±2° C.

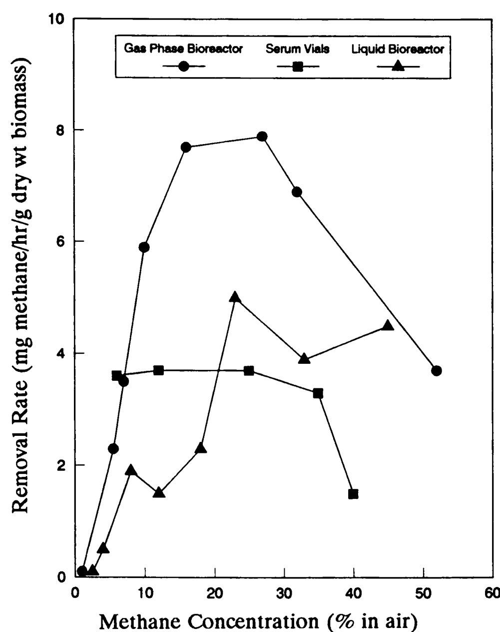

Figure 6. Comparison of methane removal rates by *Methylomonas methanica* grown in recirculating gas phase bioreactors, liquid bioreactors, and shaken serum vials at 22±2° C.

saturated and the metabolic activity of the cells in the biofilm would be the rate limiting step.

As initial methane concentrations further increased above 30%, methane removal rates fell precipitously to a rate of 3.9 mg methane hr^{-1} (g dry wt $biomass)^{-1}$ at 52% initial concentration of methane in air. This decrease in methane removal rates at higher initial methane concentrations may be a result of oxygen limitation. Since all experiments reported above were conducted with various concentrations of methane in air, oxygen levels contained in the bioreactors necessarily decreased as methane levels increased. For example, from Figure 7 it can be determined that at a 52% methane-in-air, the highest concentration tested, the oxygen concentration in the gas phase is only 10%. Since oxygen is an integral substrate in the methanotrophic oxidation of methane, oxygen may become the limiting substrate at higher methane-in-air concentrations leading to the decrease in methane oxidation rates noted in Figure 6. Thus, it is possible if the methane feed to these bioreactors where supplemented with additional oxygen, the maximum methane oxidation rates observed might be significantly increased both by driving the enzyme kinetics of the system towards zero-order and by fully saturating the biofilm. Supplemental oxygen feeds were not used in the experiments reported since there was a specific interest in exploring industrial processing of methane based exclusively on methane in air feed streams. It is apparent, however, that to fully assess the maximum rates of methane removal by methanotrophic bioreactors at higher methane feed concentrations, additional experimentation is needed with oxygen supplemented feed streams where bisubstrate kinetics and phase transport parameters with both methane and oxygen are considered as an integral part of the experimental design.

In net, within the context of the experimental procedures reported here, the gas phase bioreactors exhibited a notable advantage in maximum methane removal rates versus the more conventional shaken liquid cultures and the sparged liquid bioreactor. This advantage was 2.1 and 1.6 fold, respectively. These results indicate that the bioprocessing rates of gases and vapors with relatively low aqueous solubility may be increased by the use of gas phase bioreactors.

Preliminary experiments have also shown the processing of TCE vapors to be feasible using gas phase bioreactors. Figure 8 shows typical TCE removal rates using a gas phase bioreactor identical to those used for methane removal studies with the exception that a biofilm of *M. trichosporium* OB3b was employed in place of *M. methanica*. All data shown in Figure 8 were corrected for abiotic adsorption of TCE, and as such, represent bacterial degradation. Best removal rates were obtained between days 2 and 5 with TCE removal averaging approximately 9 $\mu g\ d^{-1}$ $bioreactor^{-1}$ during this period. It was suspected that the relatively long lag phase was a product of excessive initial methane feed concentrations since it is known that while methane serves as the source of reducing power for the generation of the NADH necessary for methanotrophic methane monooxygenase (MMO) to catalyze the degradation of TCE, it at the same time competitively inhibits TCE oxidation by the enzyme. A more successful strategy for TCE degradation using methanotrophic gas phase bioreactors may be to use a continuous, plug flow bioreactor design with very low levels of methane being fed in conjunction with the

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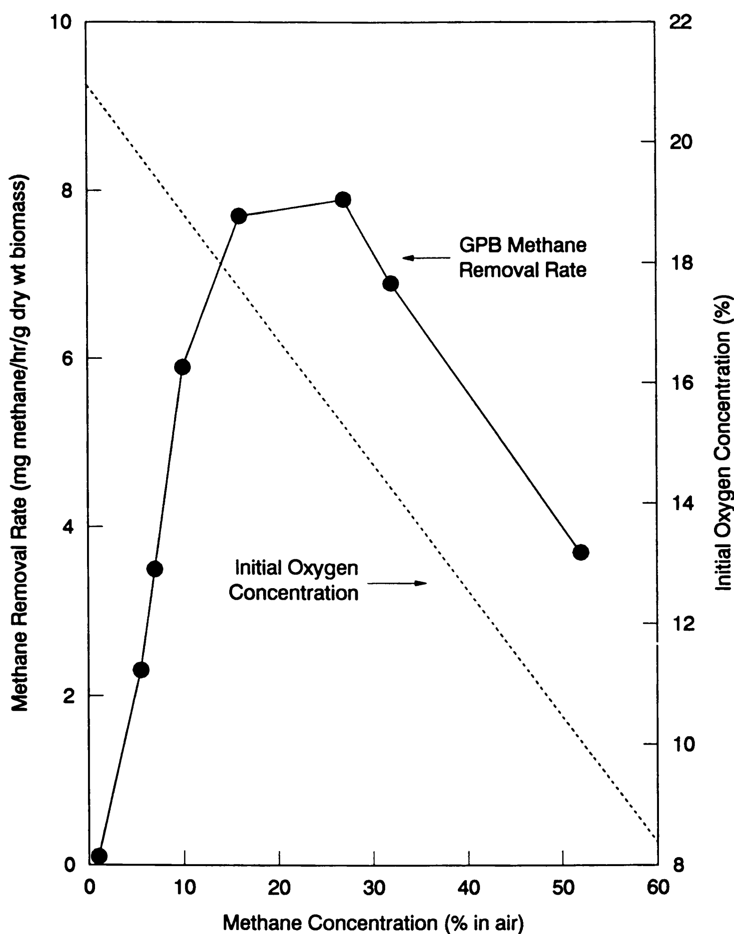

Figure 7. Comparison of methane removal rates by *Methylomonas methanica* grown in recirculating gas phase bioreactors versus initial oxygen concentrations.

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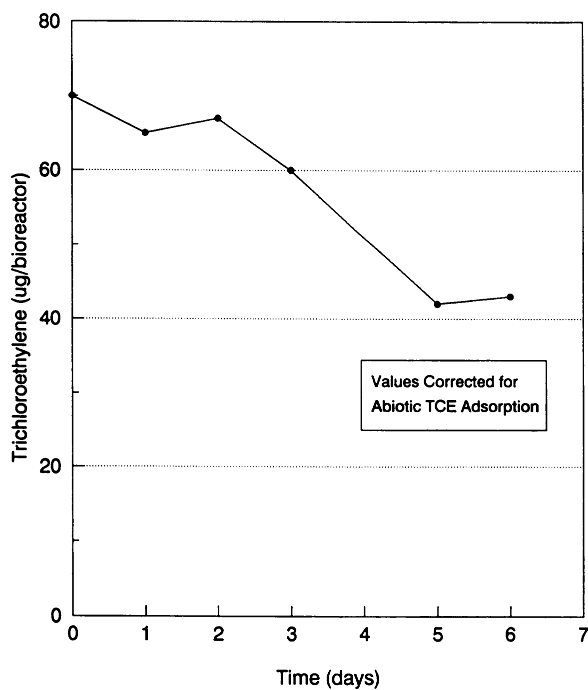

Figure 8. Trichloroethylene removal by *Methylosinus trichosporium* OB3b cultured in a recirculating gas phase bioreactor.

TCE vapors. If competitive inhibition of the MMO by methane is a factor in lowering TCE degradation rates, this may be a viable approach to lessen the inhibition and increase TCE degradation rates.

Experiments with *p*-xylene indicated that the gas phase bioreactor is capable of removing virtually all the xylene from a gas feed stream as noted in Figure 9. This figure shows a mass balance based on carbon for a gas phase bioreactor containing a stationary culture of *Pseudomonas putida*. The gas feed stream rate was 16 ml min^{-1} with a xylene feed in the gas stream of 139.9 μg min^{-1} based on carbon. The liquid medium feed rate was 1.7 ml min^{-1} and the reactor temperature was 22±2°C. Only 1.2 μg min^{-1} xylene (based on carbon) was detected in the off gas stream exiting the bioreactor, however significant partitioning of the xylene to the liquid phase in the bioreactor did occur with 74.8 μg min^{-1} xylene (based on carbon) being detected in the liquid effluent stream exiting the reactor. Only 1.2 μg min^{-1} of xylene carbon was not accounted for in the mass balance shown in Figure 9 with the remainder of the carbon being either mineralized to carbon dioxide or unidentified non-xylene organic carbon. In net, the degradation rate of the bioreactor operated under the conditions specified above was 46%. It may be possible to recirculate the liquid effluent back through the bioreactor, aerate the liquid effluent, decrease the flow rate through the column, or increase the column length to approach 100% degradation.

Based on the information conveyed in Figure 9 it is possible to calculate a volumetric productivity value for the bioreactor which can be a useful overall gauge of bioreactor conversion efficacy as well as serving as a basis for sizing scaled up reactors. The volumetric productivity value in this instance could be defined as the mass of xylene fed per unit time per unit bioreactor volume times the degradation factor (i.e. 0.46). This calculation reveals that the volumetric productivity value for the gas phase bioreactor operated under the above described conditions is 20.6 μg min^{-1} l^{-1} for *p*-xylene. This productivity value may be increased significantly by developing a secondary treatment for xylene contained in the liquid effluent as discussed above.

In conclusion, the gas phase bioreactors of the type employed in these studies appear to offer significant potential for the enhanced bioprocessing of gases and vapors. This view is supported by the data presented in this paper as well as by considerable practical experience being gained in biofiltration with soil and compost-based bioreactors (*15*), which in reality, are little more than thin film, plug flow, gas phase bioreactors. Clearly, however, more research needs to be performed in this area. A question of particular relevance that remains to be answered concerns applicability of gas phase bioreactors vs. substrate solubility. At some point, a trade-off must exist in which a gas or vapor is sufficiently water soluble that it is better processed using a conventional liquid phase bioreactor. This critical solubility point is yet to be defined.

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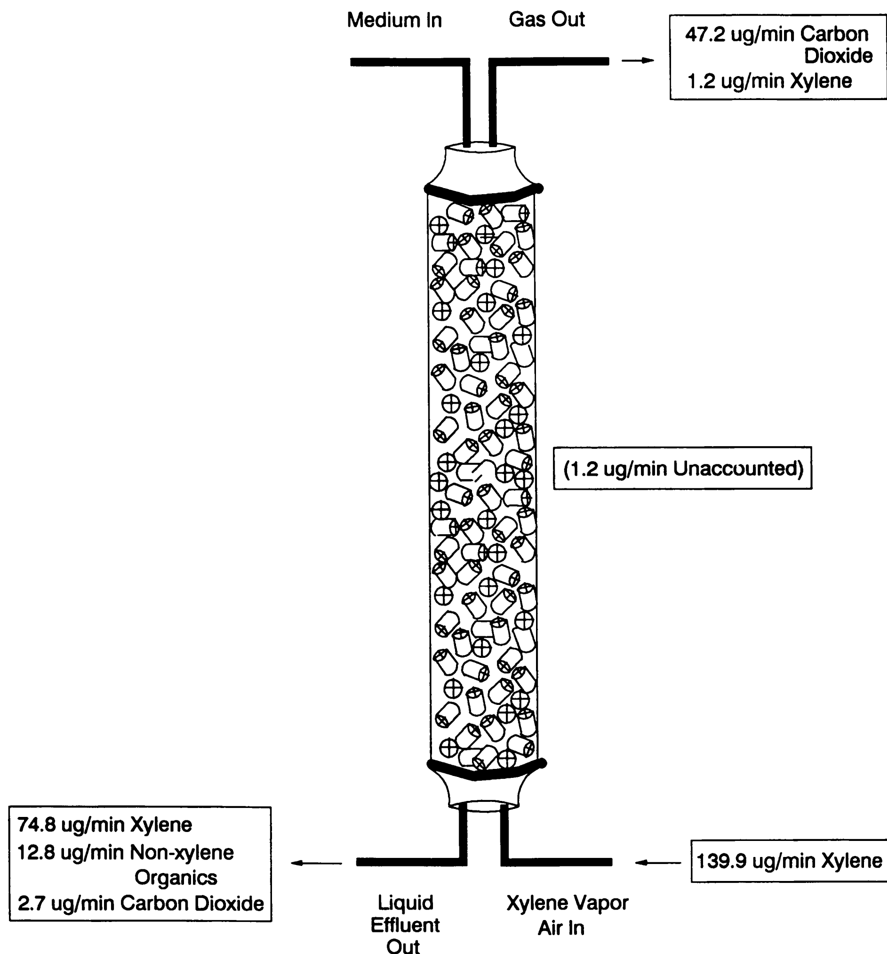

Figure 9. Mass balance of gas phase bioreactor processing xylene. All values shown as mass of carbon per unit time. Conditions were as follows: gas flow rate - 16 ml min^{-1}, liquid effluent flow rate - 1.7 ml min^{-1}, incubation temperature - 22±2° C, organism - *Pseudomonas putida*.

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