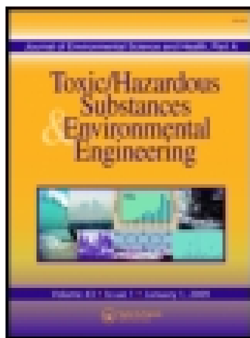

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Microbial Processing of Volatile Organics in Industrial Waste Streams

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Abstract

A strain of *Pseudomonas putida* has been isolated which tolerates and metabolizes toluene and *p*-xylene. In our laboratory, this isolate has undergone selection and adaptation and presently is able to grow under a layer of 100% *p*-xylene. From batch studies the initial rates of degradation are 1–3 mg/min/L. This strain of *P. putida* also tolerates the presence of a nonionic surfactant while still maintaining its metabolic activity. Preliminary testing using this isolate under chemostat conditions indicates that the potential for developing a bioprocess to treat these waste solvents may be possible.

Introduction

Common organic solvents are utilized for a variety of manufacturing, research, educational and health purposes and are produced in large quantities [1]. Toluene and benzene, for example, are two solvents which are produced in abundance (10^9 lbs per year). A large quantity of these solvents are not chemically altered during the process and become hazardous waste effluents. In addition to these wastes, another category of wastes, called mixed hazardous waste, was created by the regulatory agencies in 1985. As

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defined by the regulation 40 CFR 261 [2], these wastes contain both hazardous chemicals and radioactive substances. For instance, the valuable detection method employing liquid scintillation counting produces a radioactively contaminated liquid that contains an aromatic fraction, usually consisting of toluene, xylene, or pseudocumene. It is a mixed waste. Both the private sector and government facilities produce quantities of this material with an annual production of 2–3 × 10^5 gal. Although biodegradable cocktails have recently been introduced into the marketplace, a large percentage of these wastes still contain the hazardous aromatic compounds.

It is well documented in the literature [3] that benzene and its methyl substituted derivatives are metabolized by microorganisms. Previous studies have elucidated the pathways involved in the individual enzymatic steps, and many, if not all, of the genes coding for the degradative enzymes are now known.

Due to the impact of these solvents on the environment and man, many policy and regulatory changes [4] are forcing generators to consider drastic alternatives to their existing practices. Some of these alternatives involve solvent substitution, waste minimization and alternate technologies. Very few alternate technology approaches have dealt with the fact that in-line or end-of-pipe waste processing utilizing bioprocesses could make it possible to continue existing manufacturing practices. In this study we isolated microorganisms with the ability to use methylated benzene derivatives as their sole carbon source and developed a bioprocess system capable of continuously degrading these compounds.

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Methods and Materials

Microbial conditions: All studies were carried out using a basal salts medium containing the following per liter: 0.7 g KH_2PO_4, 0.3 g $MgSO_4$, 0.5 g $(NH_4)_2SO_4$ and 1.0 mL of each of the three trace minerals, $FeSO_4$-$7H_2O$, $MnCl_2$-H_2O and $NaMoO_4$-$7H_2O$. The final pH was adjusted to 6.5. All agar used was Difco Bacto Agar. All chemicals were of analytical grade or better. All cell viability studies were done on spread plates with a basal salts agar and placed in a desiccator at room temperature with an atmosphere of xylene.

Analytical conditions: Toluene and *p*-xylene were determined using a Perkin Elmer gas chromatograph (GC) fitted with a 4 ft column packed with 3% SP-1500 on Carbopack, Supelco. The injector temperature was

275 °C, column temperature 220 °C, with the flame ionization detector at 275 °C. Helium was used as the carrier gas at a flow rate of 33 mL/min. Toluene and *p*-xylene had retention times of 1.05 min and 2.47 min, respectively.

Chemostat conditions: A New Brunswick MultiGen fermenter was used as Bioreactor 1 (see Figure 1). The flask was modified with glass ports at the one liter level and one port below that level. The airstream exiting this reactor was routed directly through a water-cooled condenser and then via glass lines to the bottom of a second reactor (not shown in Figure 1) which was fitted with a medium fritted sintered glass plate. This second column reactor was 3.5 cm in diameter by 135 cm in height. All pumping was done through solvent resistant Viton tubing using Ismatec pumps.

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Sampling procedures: Mass balance data for *p*-xylene was gathered from both reactors. Liquid effluents samples were taken directly from both reactors with sterile syringes. The air samples were collected on charcoal traps over a 10-min time period. The charcoal was then placed in small scintillation vials and extracted with carbon disulfide. Charcoal extraction efficiencies of *p*-xylene with carbon disulfide ranged from 88 to 100% of the amount added to charcoal.

Results

Many industrial waste samples were collected and screened for organisms that could degrade toluene, xylenes and pseudocumene. Several isolates were chosen from flasks that had one gram of sample added to mineral salts media, pH 6.5, with 1–5 ppm of the desired aromatic compound. After several transfers were done on a basal agar medium and incubated in a desiccator with an open beaker of *p*-xylene or toluene, the pure colonies that grew up on these plates were introduced into a liquid broth with 1–10 ppm of the aromatic compound. Growth of these isolates was determined by turbidity. However, after several days the cells would die and clump.

In order to control the essential parameters of aeration and pH, a bioreactor (Figure 1) was set up and inoculated. Initially toluene or *p*-xylene was introduced via vaporization and the cells were maintained in mineral salts media in a pH range of 5.2–5.9. The cells grew to a density of 10^8–10^9 cells/mL and have survived under these continuous feed conditions for three years.

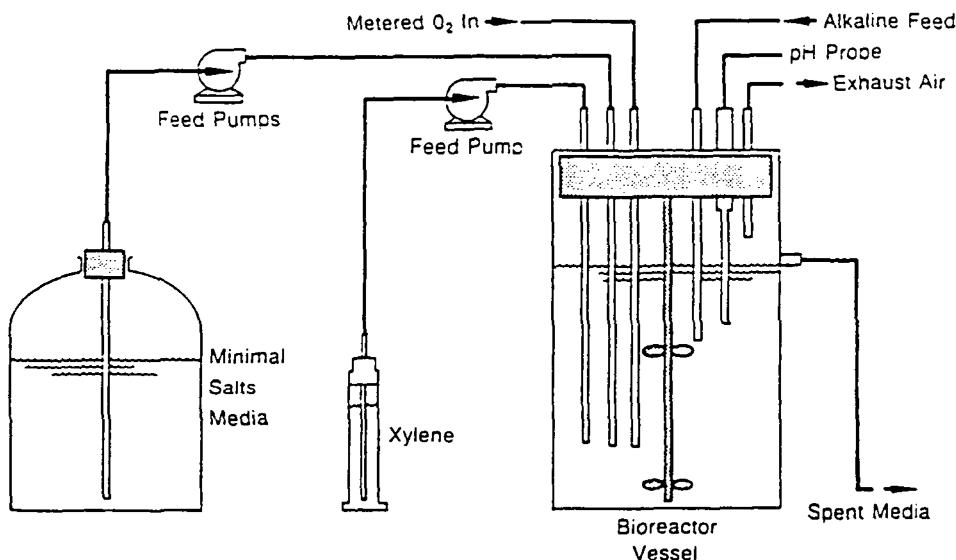

Figure 1: Schematic of bioreactor.

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Growth Studies: Selected isolates were transferred from plates, incubated in a desiccator with an atmosphere of toluene or *p*-xylene, into liquid broth tubes containing mineral salts media with 1–10 ppm of either aromatic compound. These tubes became the stock cultures for growth and utilization tests. In Figure 2, the selected isolate was followed for its ability to grow in and utilize *p*-xylene. Growth was determined by an increase in turbidity and by the disappearance of the compound as analyzed by gas chromatography. These tests were done under controlled conditions by adding 2.0 mL of the stock solution to sealed 100-mL serum bottles that contained 20 mL of sterile media saturated with the aromatic compound. The bottles were agitated by placing them inverted on a shaker table. This test was done in triplicate with the poisoned controls labelled as C-1, 2 and 3 and the samples as S-1, 2 and 3 in Figure 2. Samples were taken by a microliter syringe and immediately injected into the GC to follow the disappearance of *p*-xylene.

During the course of this work, chemostat-grown cells were observed to tolerate higher and higher concentrations of *p*-xylene. Eventually, we observed that this isolate would grow on a basal mineral salts agar with a liquid layer of *p*-xylene that is poured onto the media after streaking the plate.

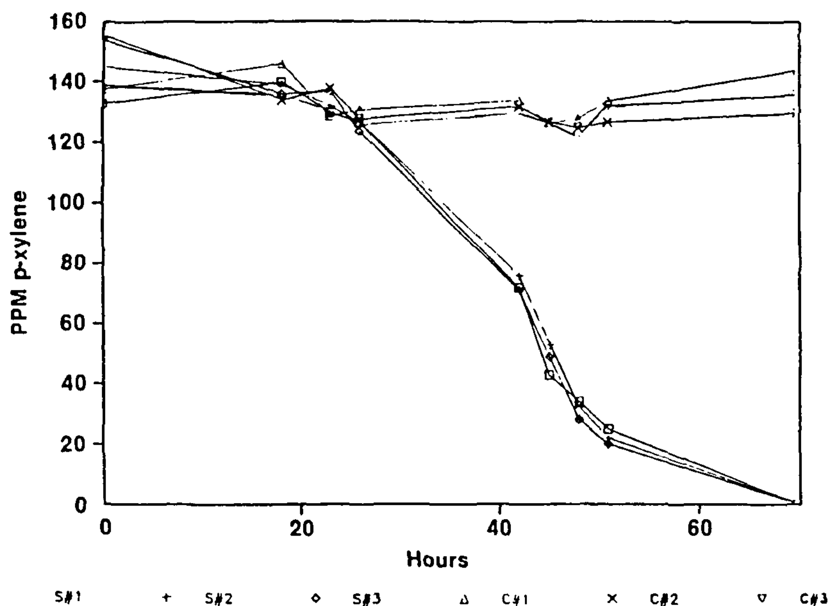

Figure 2: Degradation with a single isolate. A stock culture (2.0 mL) was introduced into a sealed 100-mL serum bottle containing 20 mL of a *p*-xylene saturated mineral salts medium.

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Batch Studies: Initially the chemostat was not configured to permit mass balance studies for the determination of rate of disappearance data. Therefore, the initial rate of disappearance was performed with cells grown in the chemostat and transferred to sealed serum bottles. Aliquots of 20–25 mL were taken from the chemostat and introduced into 100 mL serum bottles which were sealed with teflon-aluminum crimp caps. To reduce the loss of the volatile *p*-xylene from the liquid sample to the head space, the serum bottles were flushed for five minutes with a low pressure, low flow airstream that was bubbled through *p*-xylene prior to the addition of the inoculum. The serum bottles were placed inverted on a shaker table. Control serum bottles were run by placing 1.0 mL of 1% mercuric chloride in the vial prior to addition of the sample. Disappearance of xylene from the liquid was monitored over time by removing samples and poisoning them with mercuric chloride in small 1.5-mL vials. These vials were sealed as were the serum bottles and stored at

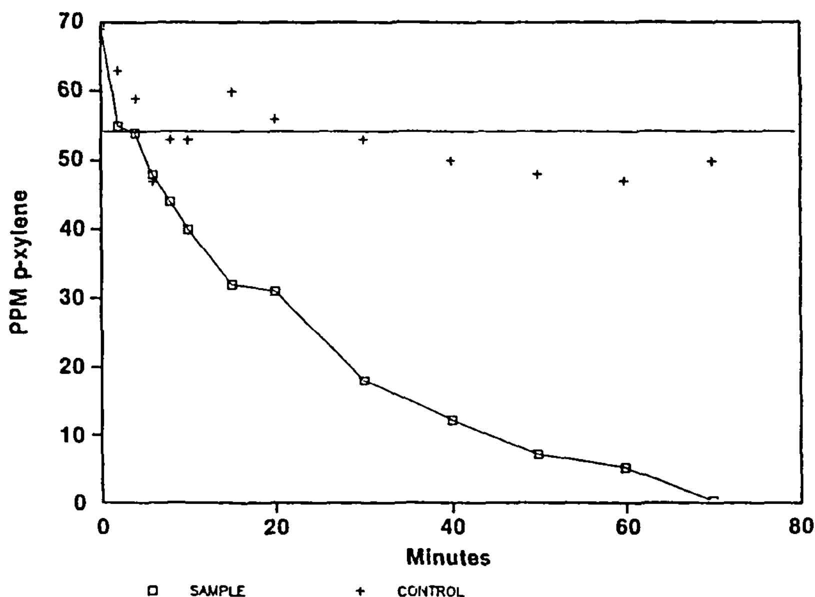

Figure 3: *p*-Xylene degradation with cells grown in the chemostat. A 20-mL chemostat sample was placed in a sealed 100-mL serum bottle. One-millimeter samples were removed and poisoned with $HgCl_2$. The control was poisoned at time zero.

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4 °C until GC analysis was done. A typical degradation curve for *p*-xylene is shown in Figure 3.

In order to determine if this isolate could maintain viability and still metabolize *p*-xylene, similar studies were done with the addition of an emulsifying agent, Triton X-100, polyethylene glycol *p*-isooctylphenyl ether. Three levels of Triton were used, 100, 1000 and 10,000 ppm. Following the initial rise of xylene after addition of the emulsifier to chemostatly grown cells, degradation occurred rapidly. Although viability was maintained in all three levels of surfactant tested, the level of 1000 ppm was selected for further testing. A triplicate test was run in which 1000 ppm of Triton was added to each serum bottle that had inoculum grown under xylene starvation conditions. In this case, each bottle also received 6 μL of *p*-xylene. Then the degradation was followed, see Figure 4.

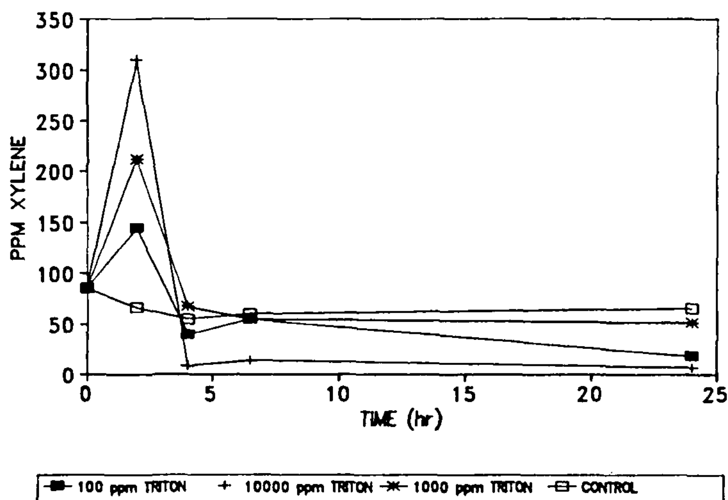

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Figure 4: Xylene degradation: 100, 1000, and 10,000 ppm Triton effect measured against control.

Continuous Studies: The bioreactor was modified to provide a closed system from which mass balance studies could be obtained, (Figure 1). All liquid and gaseous feeds and effluents were pumped into and out of the bioreactors and plumbed in glass with the joints butted to each other and held together with latex tubing. A secondary bioreactor was added as a polishing stage and in series with the first bioreactor. This column was 3.5 cm in diameter and 135 cm long. The column was fitted with a medium fritted disc near the bottom, and two strands of Ring Lace (Dodwell and Co. Ltd, Japan) were inserted in the column in hope of cell attachment. The effluents from this column were taken off the top and piped into a large holding reservoir. Although this work is ongoing, a few runs have been made at different airflow and xylene feed rates.

Initial testing was done by pumping *p*-xylene into bioreactor 1 without any emulsifier in the medium. Gas and liquid samples were taken several times during an 8-h period. Gas samples were taken to determine how much of the *p*-xylene was being vaporized in bioreactor 1 and 2. In Table 1, the results from two days of sampling are shown. The airflow was 100 mL/min to bioreactor 1, *p*-xylene and media were added at a rate of 14–25 mg/min and

Table 1: The degradation of *p*-xylene under continuous feed without emulsifier.

Airflow mL/min	Xylene feed G/24 hours	Percent degraded	Rate mg/min
100	18.7	84	11.9
100	28.8	92	18.6
100	21.6	89	12.9

Table 2: The degradation of *p*-xylene under continuous feed with emulsifier.

Airflow mL/min	Xylene feed G/24 hours	Percent degraded	Rate mg/min
100	20.6	89.6	12.8
100	20.6	86.8	12.4
100	20.6	85.0	12.2
100	20.6	87.7	12.5

0.72–0.80 mL, respectively. During this run, an average of 88% was degraded overall for three consecutive days. Thus far, a series of runs are underway with 1000 ppm of Triton X 100 in the basal salts media. The first run was done with an airflow of 100 mL/min. Table 2 contains the values obtained over the course of one day. The degradation level was maintained at 87%. A second series of runs have been accomplished with an increase in airflow. The amount vaporized from the first bioreactor was increased and the efficiency for the overall degradation decreased to 70% for three consecutive days (Table 3). Further testing in this bioreactor will be accomplished by sparging pure oxygen into the media at lower flow rates.

Discussion

Contaminated soil and water samples were chosen as sources in which microbial adaptation may have occurred. The isolate which is reported here is not only tolerant to high concentrations of toluene and *p*-xylene but also utilizes

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Table 3: The degradation of *p*-xylene under continuous feed with emulsifier.

Airflow mL/min	Xylene feed G/24 hours	Percent degraded	Rate mg/min
300	49.8	72	23
300	49.2	69	24
300	49.2	68	23

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these compounds as its sole carbon source. This isolate, also determined to be a strain of *Pseudomonas putida*, Idaho, grows under a layer of *p*-xylene on basal salts agar media. To insure that the organism is not obtaining any carbon from the agar, it is first washed with *p*-xylene before the media formulation is made. We believe this is the first demonstration of growth of cells under a layer of xylene that also can utilize it as a carbon source. In order to develop the capabilities of this organism into an economical bioprocess, a series of studies are underway. Following the selection of the isolate, a series of batch tests were run to insure that growth and degradation were taking place. As indicated in the results, growth did occur along with xylene or toluene disappearance. Although the culture did loose viability under batch conditions after a number of days, propagation under chemostat conditions with pH and aeration control has sustained viability over a period of three years without reinoculation.

Inoume and Horikoshi recently reported in Nature that an organism was isolated from soil in Japan that could tolerate high concentrations of toluene but did not utilize it for growth [5]. Their organism was identified as *Pseudomonas putida*. They also reported that it would grow on plates under a layer of toluene if a suitable carbon substrate, for example, glucose was added to the media.

Rates obtained under batch conditions using the initial rates from Figure 1 were in the range of 1–3 mg/min/L. Vecht et al. reported that an isolate of *Pseudomonas putida* grew well on toluene in batch and in chemostat conditions [6]. Although the rates were not reported, growth conditions were given. One of the applications thought to be promising for a bioprocess that could degrade xylene and toluene would be the degradation of these compounds in a mixed hazardous waste such as liquid scintillation cocktail. This waste usu-

ally contains emulsifiers. Therefore, a series of batch runs were performed which contained varying amounts of Triton X-100. Even at 10,000 ppm, this organism can maintain viability and still metabolize xylene. At the selected level of Triton X-100 (1000 ppm), the initial rate of metabolism was again about 1 mg/min/L.

Any process that might be of commercial importance must be operated on a continuous basis. The chemostat was modified to allow mass balances to be taken and yet remain aerated. Although studies are just underway, substantial amounts, 10–40 g of xylene, are being processed with a disappearance of 80–95%. Ehrhardt and Rehm, using a strain of *Pseudomonas putida* for the degradation of phenol, obtained rates of 10 mg/min/L [7]. However, in a later report by Zache and Rehm, complete degradation of phenol took 80 hours with a rate of 0.25 mg/min/L for free cells and 1.8 mg//min/L for immobilized cells [8].

Although not reported in the tables, bioreactor 2 is not functioning well and is a poor design for a polishing reactor. Only about 2–5% of the xylene that leaves the first reactor is degraded in the second. Although the cell density in bioreactor 2 is similar to that in the first, and xylene content in the liquid effluent is lower than that entering the reactor, very small disappearance percentages are observed. We believe this to be because the majority of the xylene not degraded in the first bioreactor is vaporized. The partition coefficient between vapor and aqueous phases are large, and therefore the retention time in the column for the vapor is too short to achieve the liquid solubilization necessary for microbial degradation. A packed-bed polishing reactor is now being tested.

In summary, an isolate has been identified which appears to have unusual capabilities for living in high concentrations of aromatic solvents and for utilizing these compounds as an energy and carbon source. We believe a simple bioprocess system incorporating this organism can serve as an in-line process to handle air emissions or liquid by-product effluents from industrial or laboratory processes. At present, this process development is designed to demonstrate that even a mixed-hazardous waste can be treated and, it is hoped, can be disposed of more easily than earlier processes allow under the new regulations.

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References

[1] T. Leisinger, A. M. Cook, R. Hutter, and J. Nuesch, editors. *Microbial Degradation of Xenobiotics and Related Compounds.* Academic Press, 1981.

[2] U.S. Government Printing Office. *Protection of Environmental.* CFR 40 260-299, Revised July 1, 1990.

[3] D. T. Gibson. Microbial metabolism of aromatic hydrocarbons and the carbon cycle. In S. R. Hagedom et al., editor, *Microbial Metabolism and the Carbon Cycle*, pages 43–52. Harwood Academic, Chur, Switzerland, 1988.

[4] D. Hansen. Benzene emissions ordered cut by 90%. *C&EN*, 5–6, September 4, 1989.

[5] Akira Inoue and K. Horikoshi. A pseudomonas thrives in high concentrations of toluene. *Nature*, 338:264–266, 1989.

[6] S. E. Vecht, M. W. Platt, Z. ErEl, and I. Goldberg. The growth of *Pseudomonas putida* on m-toluic acid and on toluene in batch and in chemostat cultures. *Applied Microbiology and Biotechnology*, 27:587–592, 1988.

[7] H. M. Ehrhardt and H. J. Rehm. Semi-continuous and continuous degradation of phenol by *Pseudomonas putida* p8 adsorbed on activated carbon. *Applied Microbiology and Biotechnology*, 30:312–317, 1989.

[8] G. Zache and H. J. Rehm. Degradation of phenol by a coimmobilized entrapped mixed culture. *Applied Microbiology and Biotechnology*, 30:426–432, 1989.

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