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Enhanced biodegradation of methylhydrazine and hydrazine contaminated NASA wastewater in fixed-film bioreactor

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Abstract

The aerobic biodegradation of National Aeronautics and Space Administration (NASA) wastewater that contains mixtures of highly concentrated methylhydrazine/hydrazine, citric acid and their reaction product was studied on a laboratory-scale fixed film trickle-bed reactor. The degrading organisms, *Achromobacter* sp., *Rhodococcus* B30 and *Rhodococcus* J10, were immobilized on coarse sand grains used as support-media in the columns. Under continuous flow operation, *Rhodococcus* sp. degraded the methylhydrazine content of the wastewater from a concentration of 10 to 2.5 mg/mL within 12 days and the hydrazine from ∼0.8 to 0.1 mg/mL in 7 days. The *Achromobacter* sp. was equally efficient in degrading the organics present in the wastewater, reducing the concentration of the methylhydrazine from 10 to ∼5 mg/mL within 12 days and that of the hydrazine from ∼0.8 to 0.2 mg/mL in 7 days. The pseudo first-order rate constants of 0.137 day^{-1} and 0.232 day^{-1} were obtained for the removal of methylhydrazine and hydrazine, respectively, in wastewater in the reactor column. In the batch cultures, rate constants for the degradation were 0.046 and 0.079 day^{-1} for methylhydrazine and hydrazine respectively. These results demonstrate that the continuous flow bioreactor afford greater degradation efficiencies than those obtained when the wastewater was incubated with the microbes in growth-limited batch experiments. They also show that wastewater containing hydrazine is more amenable to microbial degradation than one that is predominant in methylhydrazine, in spite of the longer lag period observed for hydrazine containing wastewater. The influence of substrate concentration and recycle rate on the degradation efficiency is reported. The major advantages of the trickle-bed reactor over the batch system include very high substrate volumetric rate of turnover, higher rates of degradation and tolerance of the 100% concentrated NASA wastewater. The results of the present laboratory scale study will be of great importance in the design and operation of an industrial immobilized biofilm reactor for the treatment of methylhydrazine and hydrazine contaminated NASA wastewater.

Introduction

Hydrazine (Hz) and its derivatives, monomethylhydrazine (MMH) and 1,1-dimethyl hydrazine (UDMH) are major constituents in a variety of rocket fuels and missile propellants. They are also widely used in the agricultural and pharmaceutical industry (Schmidt 1984). These uses have led to the inadvertent release of the chemicals into the environment and the accumulation of large volumes of industrial wastewater containing toxic levels of these hydrazine fuels. The carcinogenicity of the hydrazines to laboratory animals (IARC 1974), as well as their determined and potential hazards to humans and the surroundings have led to the concern for their fate in the environment. In addition, there is the task of finding appropriate cost-effective methods for the treatment and safe disposal of industrial wastes containing high levels of effluent hydrazine fuels.

It is customary, in many industries, and in particular in aerospace operations, to neutralize the corrosiveness of waste hydrazine by addition of calculated amounts of citric acid solution. The National Aeronautics and Space Administration (NASA) at the Kennedy Space Center in Florida, vent gaseous nitrogen (GN_2), carrying hydrazine and methylhydrazine vapors, are scrubbed with citric acid solution to absorb the hydrazines (hydrazine and methylhydrazine) in scrubber liquor. While the cleansed GN_2 is vented to the atmosphere, environmentally hazardous citric acid/hydrazine mixtures are accumulated, posing decontamination challenges. Biological degradation of these hydrazine-containing wastes is probably the most cost-effective onsite-site remediation option (Alexander 1985).

In an earlier study (Nwankwoala et al. 1999), we reported that NASA's scrubber water containing large amounts of methylhydrazine and citric acid was amenable to biodegradation in batch cultures by *Achromobacter* sp. and *Rhodococcus* sp. The microbes were found to degrade more than 50% of the methylhydrazine, citric acid and their reaction product when the NASA wastewater was inoculated in batch cultures. To check whether the process would be amenable to industrial application and as well as improve the degradation rate and efficiency, we have employed a trickle-bed reactor column with immobilized microbes on solid support. Immobilized cell cultures have been found to achieve high rates of chemical removal and tolerate harsh conditions and shock loadings (Cassidy & Trevors 1996). This paper presents the results of the enhanced degradation of the hydrazines and citric acid contaminants present in the NASA wastewater on the trickle-bed reactor column with immobilized *Achromobacter* sp. and *Rhodococcus* sp. The performance of the biofilm reactor was evaluated over a range of substrate concentrations and recycle/retention times.

Materials and methods

Bacteria source and culture conditions

Achromobacter sp. (ATCC 21910) was obtained from the American Type Culture Collection (ATTC) while *Rhodococcus* B30 and J10 strains were revived from previous isolates preserved by the Environmental Science Group at Tuskegee University. *Achromobacter* sp. was grown in ATCC Culture Medium 457 with the following constituents per liter: K_2HPO_4, 7.32 g; Ammonium tartarate, 4.6 g; KH_2PO_4, 1.09 g; $MgSO_4{\cdot}7H_2O$, 0.04 g; $FeSO_4{\cdot}7H_2O$, 0.04 g; and $CaCl_2{\cdot}2H_2O$, 0.014 g (pH 7.5). The *Rhodococcus* sp. were grown in a modified Basal Salt Media with the following composition per liter: K_2HPO_4, 1.6 g; KH_2PO_4, 0.4 g; NH_4NO_3, 0.5 g, NaCl, 0.1 g, $MgSO_4{\cdot}7H_2O$, 0.2 g; $CaCl_2{\cdot}2H_2O$, 0.025 g, $FeCl_3{\cdot}6H_2O$, 0.003 g, glycerol, 0.1 mL (pH 7.0). All the chemicals, except ammonium tartarate, were from Fisher Scientific and were of Certified A.C.S. grade. Ammonium tartarate was obtained from Aldrich Chemicals. All media and handling equipment were sterilized by autoclaving at 121°C for 15 minutes.

The culture flasks, each containing ~100 ml growth cultures, were agitated on an orbital shaker at 200 rpm and maintained at their respective optimal temperatures (30°C for *Achromobacter* sp. and 28°C for *Rhodococcus* sp.) in the laboratory's environmental chamber. The design ensured a high surface-to-volume ratio and allowed sufficient diffusion of oxygen to the bottom of the flasks. Periodic serial transfer of microbes to fresh media maintained the cultures. Purity and phenotypic behaviors were ensured by continuous abbreviated characterization of each subculture. Microbial growth in the batch cultures was monitored by UV/visible spectrophotometry, turbidimetry and total organic carbon measurements.

Harvesting and purification of cell suspension

Concentrated cells for biodegradation experiments were harvested from the cultures by differential centrifugation. Cultures were harvested during their period of maximum stability and viability, characterized by the mid-logarithmic growth phase. Cells were harvested by filtering the cultures of each strain through Whatman No. 1 filter paper to remove any precipitate that may be present. The filtrate was then centrifuged at 10 000 g for 10 minutes (Fischer-Marathon Model 22KR refrigerated super-speed centrifuge). The resulting cell pellet was washed twice by re-suspending in about 10 ml of sterile KH_2PO_4/K_2HPO_4 buffer (pH = 7.2) and centrifuged. The washings were repeated for three more times. The cell harvestings were all done aseptically.

The trickle-bed reactor

The trickle-bed reactor consists of cylindrical plexiglass column (0.9 m × 10 cm I.D) packed with coarse

sand grains as biomass support media. The sand were sieved to obtain the appropriate grain sizes (usually 6–8 mesh, ∼3 mm), rigorously washed, acid-treated for 24 hours and rinsed with distilled water. They were then autoclaved at 103 kpa and 121°C for 90 minutes, incubated for 24 hours, and then autoclaved for additional 2 hours. This treatment removed possible organic and inorganic constituents that could interfere with the on-column biodegradation reactions and ensured that the biomass support materials remained chemically and biologically inert.

Combined harvested biomass estimated at >1500 mg (wet weight) cells ml^{-1}, were used to inoculate slurry made from the solid support materials and the growth media for the particular microbe in use. The microbes were shaken and incubated on the slurry for 24–48 hours in the laboratory's environmental chamber. This period was found adequate for the initiation of microbial growth and immobilization on and within the inert support particles. The growth slurry was then packed into the trickle-bed reactor and the initial liquid media drained off. The column was continuously fed (steady-state) with prepared NASA wastewater from a feed reservoir. A Rabbit peristaltic pump (RAININ Instrument Co. Inc.) which was on-line with the reactor-bed and the wastewater feed stock, drove flow through the bed.

The laboratory scale reactor had a bed-volume of approximately 400 mL when packed with sand grains of 6–8 Mesh size (2.36–3.35 mm). The reactors were operated with one bed-volume of feedstock during each run with the inlet and outlet flow rates set approximately equal to each other. Flow rates ranged from 20 to 90 mL/hour and at any particular time during the continuous flow, one-half of the feedstock volume was in the reactor column while the other half remained in the reservoir flask. Sterile air was continuously pumped into the reactor, at a flow rate of approximately 85 mL/min, in a counter-flow direction to the feedstock flow to maintain sufficient aerobic conditions. The transfer lines were all made of Teflon.

The feedstock was composed of NASA wastewater injected with some inorganic nutrients. The test microorganisms had already been shown to derive their carbon and nitrogen needed for their growth from the organic contaminants present in NASA wastewater while the inorganic nutrients acted as sources of trace elements necessary for cell growth and development. The feed reservoir was also continuously aerated with the help of an aquarium air pump (Tetra Second Nature Inc.) to ensure that adequate oxygen

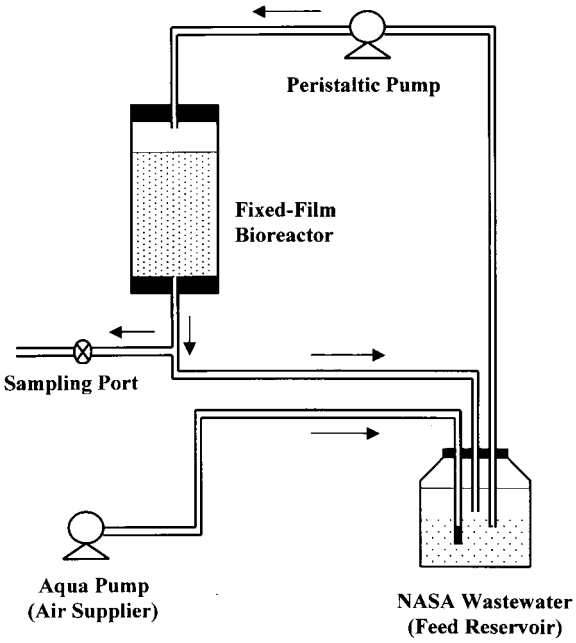

Figure 1. Schematic diagram of the laboratory trickle-bed bioreactor set-up.

was supplied to the microbes in the column and also to homogenize the feedstock. The pH of the feed reservoir was maintained at between 7.0 and 7.5. In the case of the *Rhodococcus* sp., the NASA wastewater was also spiked with dilute solution of glycerol (1% vol/vol), which was found to enhance the biodegradation of NASA wastewater by these microbes during our batch culture investigations (Nwankwoala et al. 1999). Both the inlet and outlet of the reactor column were fitted with 0.45-m membrane filters to prevent the entrance and exit of microbes in and out of the column during operation. Feed samples were replaced with new stock once analysis of collected samples revealed limiting concentrations (the concentration at which further biodegradation becomes insignificant) of the organic compounds present in the wastewater. A schematic diagram of the trickle-bed reactor assembly in its operational mode is illustrated in Figure 1.

During operation of the trickle-bed reactor, the flow and recycle rate as well as the substrate concentration were varied to investigate their effect on the microbial degradation of contaminants present in the NASA wastewater. The reactor was set-up in the laboratory's environmental chamber maintained at a temperature of 28–30°C. Control experiments were conducted in which the microbes were omitted in the reactor column. Furthermore, sorption and desorption

properties of the organics in the wastewater with the sand grains were studied in batch systems to ascertain that the on-column removal of the organic compounds were of microbial origin.

Chemical analysis and biodegradation monitoring

Samples were collected from the sampling port and from the feed reservoir, at intervals ranging from every 2 hours at the beginning of the biodegradation to once a day as the degradation rate slowed down. The samples were chemically analyzed to determine the degree of biodegradation of the contaminants present in the wastewater. Collected samples were first centrifuged (14 500 × g) and appropriately diluted before been analyzed by GC-MSD, HPLC, TOC and UV/visible. The UV/visible method was used for the analysis of residual hydrazine and methyl hydrazine at 455 nm after derivatization with *p*-dimethylaminobenzaldehyde (Hach 1992). A Rosemount-Dohrmann DC-180 TOC analyzer utilizing the persulfate oxidation method was used for the total organic carbon analysis while a Hewlett Packard Model 1050 HPLC system with a variable wavelength UV detector was used for the HPLC analysis. Reverse-phase HPLC separations of the collected samples were accomplished on an ODS-Hypersil C_{18} column (25 cm × 4.6 mm I.D). Programmed mixtures of 0.25 M H_2SO_4, methanol and acetonitrile served as the eluent. The wavelength of detection was at 254 nm.

Upon completion of the degradation experiment, the samples were adjusted to pH < 3 and extracted with dichloromethane (DCM) for possible acidic metabolites. Further extractions were done after adjusting the pH to >11 for possible base/neutral fractions. The concentrated DCM acidic extracts were derivatized with diazomethane to yield possible methyl ester derivatives. The DCM extracts were analyzed by GC/MSD after concentration in a rotary evaporator. The GC analysis was conducted with a HP 5890 gas chromatograph coupled to an HP 5970 Mass Selective Detector (MSD). The injection and detector temperatures where set at 250°C while the oven was temperature programmed (100°C held for 2 minutes, then raised to 210°C at 7°C min^{-1}). A HP-5MS column (low breed 5%-diphenyl-95%-dimethylsiloxane copolymer), 0.25 μm ID, 0.25 mm (film thickness) and 30 m long was used for the analysis.

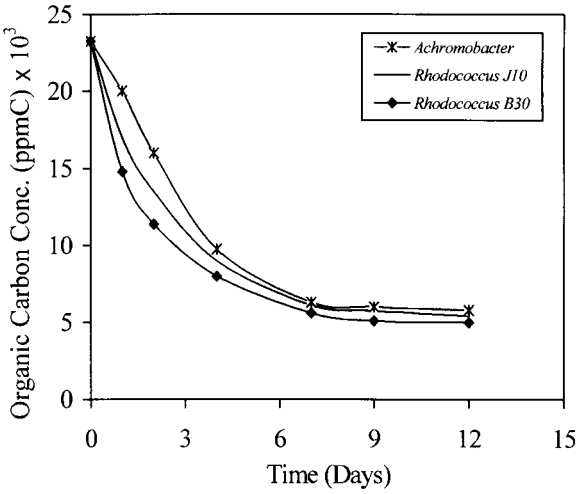

Figure 2. Typical variations in total organic carbon concentration during the degradation of NASA wastewater by the three microbes in the trickle-bed reactor column.

Results and discussion

Total organic carbon depletion

Figure 2 shows typical curves for the degradation of total organic carbon present in the NASA wastewater following continuous passage through the trickle-bed reactor. The average flow rate in these experiments was 55 mL/hour. The data are from the wastewater batch with predominantly methylhydrazine residues. The three microbes were very effective in the mineralization of the carbon constituents of the wastewater. The kinetic plots show an initial degradation reaction that proceeded at a very fast rate followed by a slower degradation process that leads to an almost limiting carbon concentration.

For the three microbes, the greater part of the microbial degradation for each new feedstock took place within the first 4 days. During this period, more than 60% of the initial organic carbon content of the NASA wastewater was consumed. Microbial activity on the depleted feedstock after 6 days is very slow and leads to little additional degradation. The level of organic carbon remained constant after about 9 days when 70–75% of the initial concentration has been consumed. It is at this point that the feedstocks were replaced with flesh samples.

Although hydrazine itself did not constitute a source of organic carbon for the microbes, the rate of removal of organic carbon was found to be much faster in wastewater with predominantly hydrazine contaminant than that containing mostly methylhydrazine.

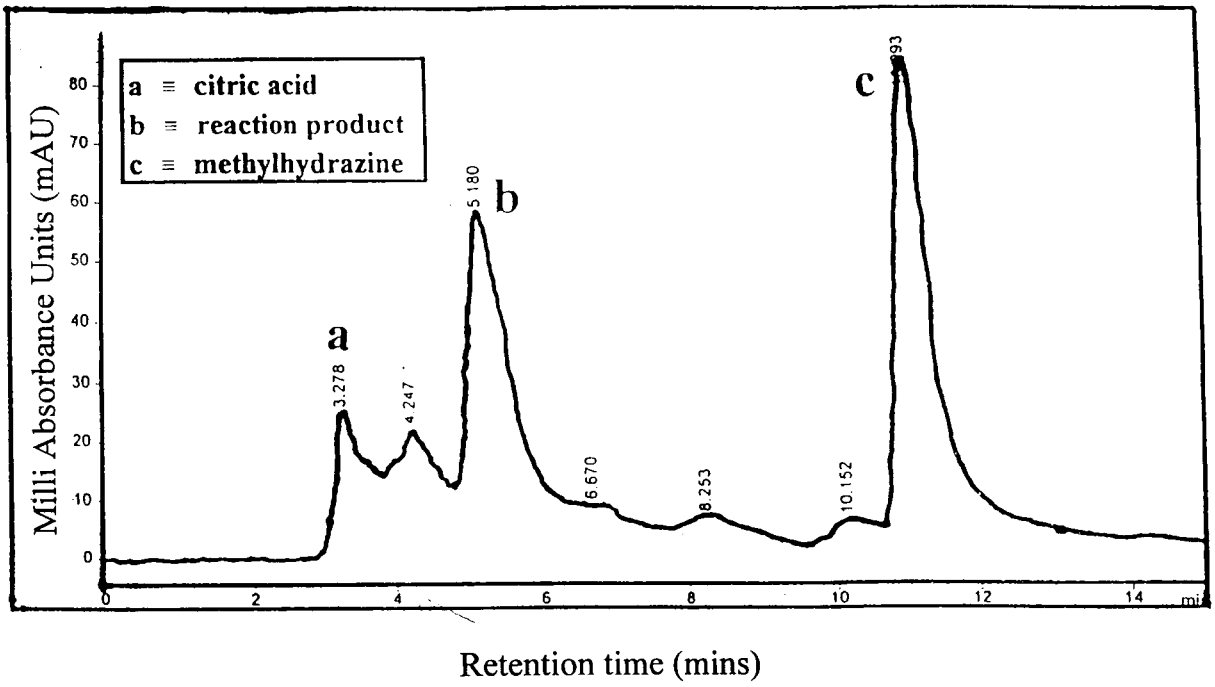

Figure 3. HPLC chromatogram of NASA industrial fuel wastewater.

For the wastewater containing hydrazine, more than 60% of the initial organic carbon was degraded within the first 2 days and the limiting concentration (less than 20% organic carbon remaining) for the biodegradation was achieved after only about 4 days.

HPLC analysis

The HPLC analysis of the NASA wastewater samples is shown in Figure 3. The chromatogram is from a methylhydrazine-contaminated wastewater. Analysis of pure samples of citric acid and methylhydrazine as well as their mixtures led to the assignment of the bands as shown in Figure 3. Wastewater samples collected when hydrazine was the major fuel in use were similarly resolved by HPLC. Our previous investigation revealed the formation of an abiotic product by the reaction of citric acid and hydrazine. An "azo" carbonyl derivative of the citric acid, consistent with the spectral data obtained from the investigation was proposed as the possible product (Nwankwoala et al. 1999). The study also showed that the industrial wastewater contains other minor species that are evident in the HPLC chromatogram, in addition to methylhydrazine (or hydrazine), citric acid and the reaction product.

Degradation curves for each of the three major contaminants present in the wastewater were obtained from the reduction in the peak areas of the chromatographic bands of samples collected at various times during the degradation. Typical degradation plots are shown in Figures 4(a–d) for methylhydrazine, hydrazine, citric acid and the reaction product of the hydrazines and citric acid respectively. Absolute concentrations of methylhydrazine, hydrazine and citric acid were obtained by analyzing standard samples of the compounds under the same conditions while those of the ascribed product were evaluated from carbon balancing based on the postulated structure (Nwankwoala et al. 1999) and the TOC results. Since the outlet of the trickle-bed reactor had a 0.45-μm filter to prevent bacteria from exiting the reactor column, it is assumed that essentially all degradation occurred within the biofilm. The methylhydrazine and hydrazine concentrations were confirmed by UV/visible measurements.

Degradation of methylhydrazine, hydrazine, citric acid and their reaction product

As the NASA wastewater passes through the reactor column, the microorganisms grown as fixed-films in the support utilize it as source of carbon, nitrogen

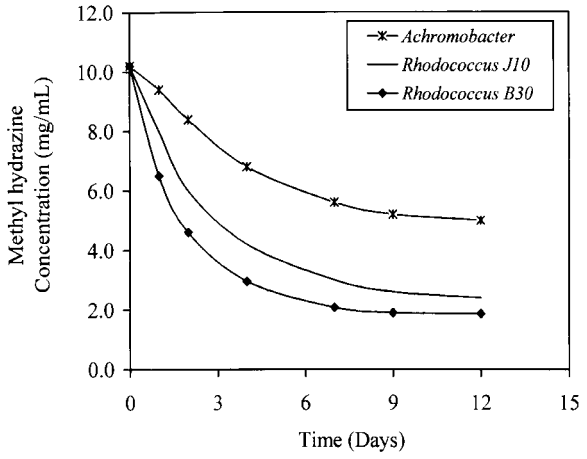

Figure 4a.

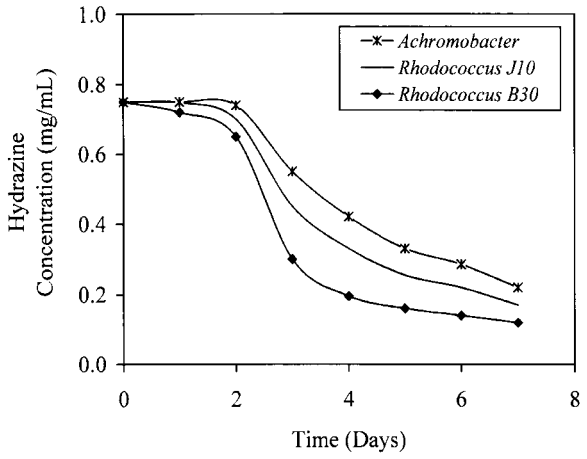

Figure 4b.

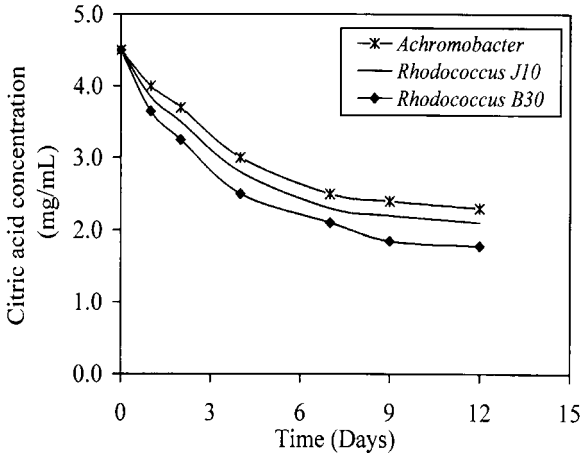

Figure 4c.

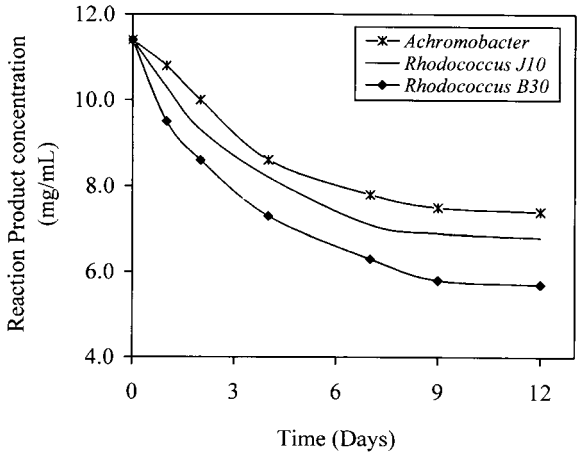

Figure 4d. Changes in (a) methylhydrazine, (b) hydrazine, (c) citric acid and (d) reaction product concentration following the degradation of the wastewater by the three microbes in the trickle-bed reactor column.

and other trace minerals needed for their growth. Repeated passage through the trickle-bed reactor greatly enhanced the biodegradation of the organic carbons in the NASA wastewater compared to the rates observed with incubations in batch cultures for the microbes (Nwankwoala et al. 1999).

From the HPLC analysis plots shown in Figures 4(a–d), both *Achromobacter* sp. and *Rhodococcus* sp. were effective in the degradation of methylhydrazine within the trickle-bed columns with the *Rhodococcus* B30 showing the most activity. The degradation of methylhydrazine was rapid during the first 4 days and gradually slowed down to a limiting concentration. By the end of the 12th day, the *Achromobacter* sp. and the *Rhodococcus* B30 had degraded 50 and 74% respectively, of the methylhydrazine, relative to the starting concentrations of about 10 mg/ml.

Wastewater samples that were predominantly contaminated by hydrazine residues were more efficiently degraded within the column with an overall degradation of almost 80% (*Rhodococcus* B30) of the initial hydrazine within the first 3 days. The limiting concentration for the degradation of hydrazine within the column reactor was less than 0.010 mg/ml (10 ppm, wt/vol) after a total hydraulic retention time of 10 days in the reactor. It should be noted that different batches of the NASA wastewater contained methylhydrazine or hydrazine as the predominant contaminant hence different substrate reservoir were used for the experimental runs illustrated in Figures 4(a) and 4(b). The difference in the degradative abilities of the microorganisms towards hydrazine and methylhydrazine

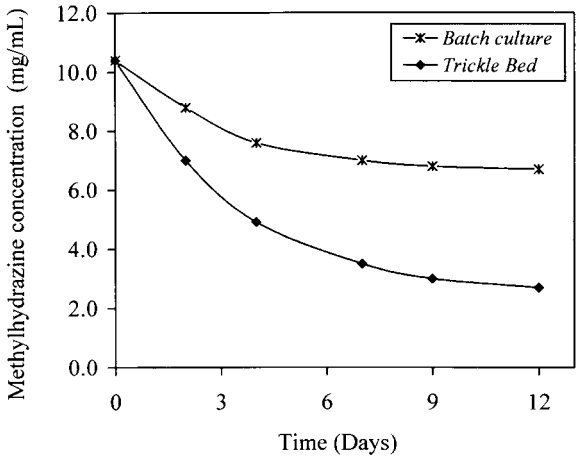

Figure 5. Batch culture versus trickle-bed reactor degradation of methylhydrazine by *Achromobacter* sp.

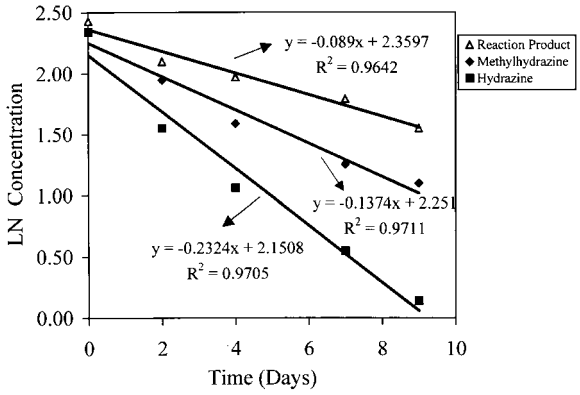

Figure 6. Pseudo first-order kinetic plots for biodegradation of methylhydrazine, hydrazine and reaction product in the trickle-bed reactor by *Achromobacter* sp.

is attributed to the greater toxicity of methylhydrazine to microbes (Kane & Williams 1983). In batch culture experiments, the maximum amount of methylhydrazine degraded was 40% of the initial concentration and this occurred over a period of 20 days indicating that the trickle-bed column provide a more efficient way of bioremediating the NASA scrubber water (Figure 5).

The *Rhodococcus* B30 was found to be most effective in the degradation of citric acid relative to the *Achromobacter* sp. in the trickle-bed columns. The microbe was able to degrade more than 40% of the residual citric acid present in NASA wastewater within a period of 4 days when continuously passed through the trickle-bed column reactor.

The kinetics of the degradation of methylhydrazine, hydrazine or the reaction product can be expressed with the second-order rate equation:

$$\mathrm{d}[\mathrm{C}_i]/\mathrm{d}t = k_2[\mathrm{X}][\mathrm{C}_i], \qquad (1)$$

where $[\mathrm{C}_i]$ is the concentration of the contaminant in question; [X] is the microbial concentration; k_2 is the second-order rate constant; and t is the time.

Since the biomass concentration effecting biodegradation is relatively high, it can be assumed to remain essentially constant during the course of the experiment, hence the kinetics can be approximated by a pseudo first-order rate equation:

$$\mathrm{d}[\mathrm{C}_i]/\mathrm{d}t = k_{\mathrm{obs}}[\mathrm{C}_i], \qquad (2)$$

where k_{obs} is the pseudo first-order rate constant.

The pseudo first-order integrated rate plots for the degradation of the contaminants in the trickle-bed reactor are shown in Figure 6. Since the concentration of hydrazine in the hydrazine containing wastewater was relatively lower than those of methylhydrazine in the methylhydrazine contaminated water, the concentration of hydrazine in Figure 6 was normalized to that of methylhydrazine for easy comparison. Also the experimental data points during the lag phase observed in the microbial degradation of hydrazine were discarded in the pseudo first-order kinetics analyses. The results are also presented in Table 1 where they are compared with those obtained in batch cultures. The pseudo first-order rate constants of 0.137 and 0.232 day^{-1} were obtained for the removal of methylhydrazine and hydrazine, respectively, in wastewater in the reactor column while removal rates in batch cultures were 0.046 and 0.079 day^{-1} respectively. These results demonstrate that the continuous flow bioreactor afford greater degradation efficiencies than those obtained when the wastewater was incubated with the microbes in growth-limited batch experiments. They also show that wastewater containing hydrazine is more amenable to microbial degradation than one that is predominant in methylhydrazine in spite of the longer lag period obtained with the hydrazine contaminated wastewater.

The degradation of the reaction product between citric acid and methyl hydrazine in the bioreactor was found to be comparable to those obtained in batch cultures and in fact exhibited a marginal decrease with *Rhodococcus* J10. About 60% of the reaction product was removed in the column degradation before the onset of the limiting concentration while about

Table 1. Pseudo first-order degradation rate constants for methylhydrazine, hydrazine and the reaction products in batch and trickle-bed continuous bioreactor by *Rhodococcus* B30

	Pseudo first-order rate constants (day^{-1})		
	Methyl hydrazine	Hydrazine	Reaction product
Trickle-bed reactor	0.137	0.232	0.089
Batch degradation	0.046	0.079	0.105

65% degradation was achieved in the batch cultures. The determined pseudo first-order degradation rate constants for the reaction product in the trickle-bed and batch experiments were 0.089 and 0.105 day^{-1} respectively.

The HPLC chromatograms taken during the microbial degradation did not reveal any new bands that could be attributed to metabolites of the degraded compounds. Also GC/MSD analysis of the extracted and derivatized samples gave no peaks that could be assigned to degradation products. Consequently, the degradation of the organic compounds present in the scrubber water by the organisms used in this experiment is presumed to lead essentially to complete mineralization.

None of the control experiments (without the microbes) yielded any significant removal of the organics in the wastewater confirming that the removals were biologically facilitated. Sorption and desorption studies also showed that the sand grains used as support media had negligible sorption capacity for the organic compounds present in the scrubber water.

Effect of substrate concentration

The supplied NASA wastewater was used at various levels of dilution in the degradation studies. The dilutions were done for two main reasons. The first was to ensure that the levels of the chemical contaminants present in the full-strength NASA wastewater did not pose toxic risks to the microorganisms and secondly to check for the possible existence of an optimum concentration at which the activities of the microbes were maximized.

Experiments were conducted using 5, 10, 25 and 100% (vol/vol) aliquots of the concentrated wastewater. Distilled water was used for the dilution. The degradation of methylhydrazine at various concentration levels of the wastewater is shown in Figure 7.

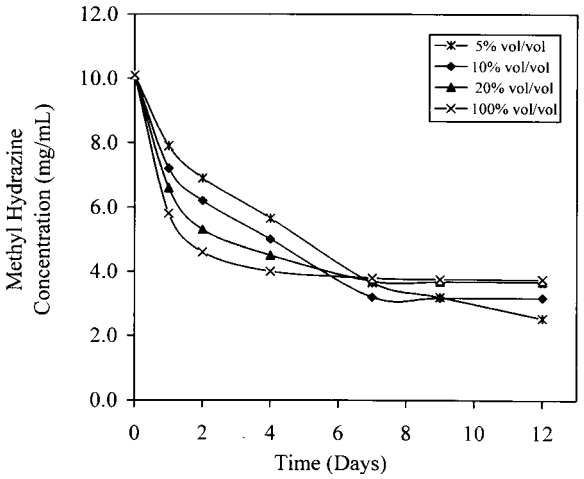

Figure 7. Effect of substrate concentration on the degradation of methylhydrazine by *Achromobacter* sp.

The plotted concentrations have been normalized to the concentration of methylhydrazine in the undiluted wastewater for ease of comparison.

The results show that microbial degradation of the hydrazines can take place at all concentration levels of the NASA wastewater. Thus, even the full-strength (undiluted) NASA wastewater does not pose toxic risks to the microbes and did not inhibit microbial degradation. From the slopes of the initial portions of the degradation curves, it is obvious that the greater the concentration of the NASA wastewater, the faster the initial rate of the biodegradation. However, the limiting concentration becomes lower as the substrate becomes more dilute. This behavior is again associated with the toxicity of methylhydrazine to the microbes. Nevertheless, the larger limiting concentration that were observed for the highly concentrated feedstock is more than compensated by the initial faster degradation rate for these concentrations. It will therefore be advantageous to apply the biological treatment process to the full-strength NASA wastewater without dilution.

It was however necessary to pre-condition the column with dilute aliquots of the wastewater before the passage of the full-strength NASA wastewater. It was observed that the immobilized microbes in the trickle-bed media died immediately upon exposure to the 100% (undiluted) wastewater if the columns were not pre-conditioned with serially diluted strengths of the wastewater, an incident we termed a “concentration shock-effect”. Both NASA wastewater types containing predominantly hydrazine and methylhy-

drazine contaminants exhibited this effect when used undiluted without prior column conditioning. It therefore became necessary to pre-condition a packed and microbial immobilized column with serial dilutions of the substrate before subjecting the microbes to the degradation of wastewater containing highly concentrated contaminants.

Effect of substrate recycle rate

The variations of methylhydrazine concentrations with number of recycles during trickle-bed biodegradation of the wastewater are illustrated in Figure 8. The recycle rate is the number of circulation per given time and is essentially identical to the flow rate. Keeping the bed volume constant, the number of cycles per given time is directly increased by increasing the flow rate.

The trickle-bed reactor used for this work was designed in such a way that both the flows in and out of the reactor can be manually controlled. These flow rates were set as much as possible to be equal to each other. In the quasi steady-state mode, the cycle is a dimensionless quantity that denotes the number of times the substrate has passed through the bed after a given period of time. With a constant bed-volume and grain size, simply adjusting the flow rate varies the number of cycles per time. The number of cycles (N_c) at any given time during a run is calculated as follows:

$$N_c = \frac{D_t}{T_p}, \tag{3}$$

where D_t is the duration of the run at any time t (minutes); T_p is the period (minutes); time for one cycle given by

$$T_p = \frac{B_v}{f_r}, \tag{4}$$

where B_v is the bed volume; volume of the substrate that fills a trickle-bed column (ml); f_r is the volumetric flow rate through the assembly loop (ml/min).

The flow rate used for the results in Figure 8 was 60 mL/hour. Flow rates of 20, 40 and 90 mL/hour were also utilized in studying the substrate recycle rate and they all afforded identical results. It is apparent that most of the biodegradation of the methylhydrazine occurs within the initial cycles of the wastewater through the column. It can also be deduced from Figure 8 that for a given D_t, degradation is faster with smaller T_p (larger f_r). The implication of this, is that the degradation of the contaminants through the column can be

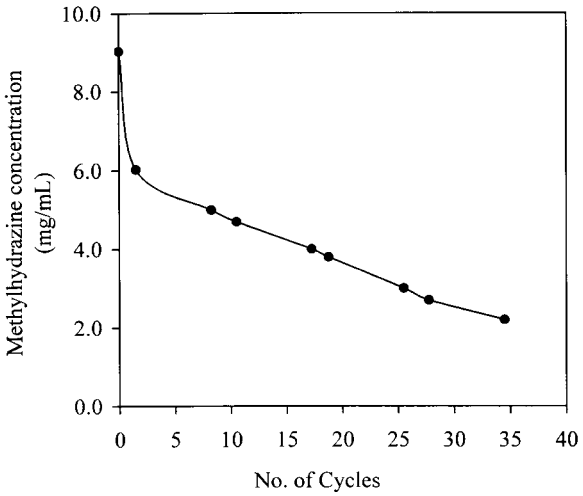

Figure 8. Effect of substrate recycle rate on the degradation of methylhydrazine in the trickle-bed reactor by *Rhodococcus* B30.

enhanced by increasing the flow rate and hence decreasing the overall time for the degradation of a given batch of feedstock. In the trickle-bed reactor system used for this study, the maximum tolerable flow rate was 90 mL/hour. In any case, excessive slow flows are not advisable in trickle-bed columns as they create non-uniform distribution of microbes. On the other hand, high hydrodynamic shear rates dislodge the immobilized cells from the support particles (Karamanev & Samson 1998). The upper flow rate is also limited by the ability of the substrate to permeate through the column bed which is determined by the grain size and hence the porosity of the support media.

Conclusion

Our results suggests that NASA wastewater containing methylhydrazine and hydrazine contaminants can be degraded more efficiently in biofilm reactors than in batch culture systems. Methylhydrazine and hydrazine degradation pseudo first-order rate constants in the biofilm reactor were 0.137 day^{-1} and 0.232 day^{-1} which were about 3 times higher than those obtained in batch cultures using the same concentration range. The results of the present laboratory scale study will be of great importance in the design and operation of an industrial immobilized biofilm reactor for the treatment of methylhydrazine and hydrazine contaminated NASA wastewater. The major advantages of the trickle-bed reactor over the batch system include very high substrate volumetric rate of turnover, higher rates

of degradation and tolerance of the 100% concentrated NASA wastewater. Also the fact that the biological transformation of the organic compounds leads to mineralization makes the process very attractive. The laboratory scale bioreactor was operated at flow rates of 20–90 ml/hour and the microbes maintained activity over several months. To prevent *concentration shock effect*, freshly prepared reactors were serially conditioned with 5, 10 and 15% (vol/vol) wastewater for 12 hours each before commencement of experiments involving the full strength wastewater. The reactor was operated at temperatures of 28–30°C and pH of 7.0–7.5 which provided optimal growth conditions for the microbes used.

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