Journal of Industrial Microbiology & Biotechnology (1997) 18, 293–296

Removal of nitrobenzene vapors by a trickling air biofilter

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A stable microbial consortium that grew on nitrobenzene (NB) as its sole source of carbon, nitrogen and energy and liberated excess nitrogen as ammonia, was immobilized on a perlite-packed trickling air biofilter. On a sustained basis, the biofilter removed 50 g NB m^{-3} packing h^{-1} and its operation at pH 8.7 resulted in ammonia stripping, making pH and salinity controls unnecessary. Low maintenance and stable performance during 4 months of continuous operation invite the scale-up of this biofilter for control of NB emissions.

Keywords: nitrobenzene; biofiltration; emission control

Nitrobenzene (NB) is widely used in the manufacture of explosives. This has left numerous manufacturing site soils and sediments polluted with NB and other nitroaromatic residues. NB is also used in the manufacture of aniline, as an industrial solvent and as a precursor of dyes and other synthetic products such as herbicides [7]. For these diverse uses, NB is produced in the USA on the scale of half a million metric tons per year [15]. NB is relatively toxic and persistent in the environment and is listed as a priority pollutant by the US EPA [14].

Nitro groups on an aromatic ring have an electron-withdrawing effect, thus impeding electrophilic attack by microorganisms on the ring structure [5,13]. One or more nitro groups on an aromatic ring tend to increase the stability of aromatics against microbial degradation, at least under aerobic conditions [6]. The first degradation pathway of nitrobenzene was elucidated by Nishino and Spain [8] and a comprehensive review of NB degradation was provided in a recent volume dedicated to the metabolism of nitroaromatic compounds [12].

Since NB is highly volatile and, without appropriate scrubbing technology, has the potential for becoming an atmospheric pollutant, biofiltration of NB may find use in the control of emissions during manufacture, as well as in bioventing of contaminated soils. For these purposes, we enriched a microbial consortium under NB vapor and investigated NB utilization by this consortium. Subsequently, we immobilized this consortium in a trickling air biofilter and assessed its potential for the treatment of NB vapor emissions.

Experimental

Enrichment and characterization of a NB-utilizing consortium

For enrichment of NB-degrading microorganisms, a 1-L sealed Erlenmeyer flask with 50 ml of mineral salts medium [2], modified by omitting its NH_4Cl component, was used. Nitrobenzene served as the sole carbon, energy and nitrogen source for the enrichment. An industrial sewage sludge not specifically exposed to NB was used as inoculum (2%, v/v) and NB was added at 12 mg L^{-1}. The flask was aerated daily and NB was added to the flask when there was no droplet of NB visible in the liquid phase. After several weeks of enrichment at 28°C with shaking, a stable microbial consortium developed. Its dominant members that grew on mineral agar plates in the presence of NB vapors were characterized by morphology, staining and the API Rapid NFT identification system (Sherwood Medical, Plainview, NY, USA).

Quantitative experiments for NB use were conducted in 1-L flasks sealed with Teflon-wrapped stoppers. These stoppers had aeration ports closed with stainless steel syringe valves (Popper & Sons, New Hyde Park, NY, USA) and a Teflon-faced septum for headspace sampling by a gas-tight syringe (Hamilton, Reno, NV, USA). The flasks received 100 ml of N-free mineral medium plus 6 mg NB, and were inoculated with the consortium. Incubation was with rotary shaking (200 rpm) at 28°C. The removal of NB from the headspace of the flasks was monitored. When the added NB was completely removed, the flasks were aerated and liquid samples were taken for protein, nitrite and ammonia determination. The flasks then received additional NB (6 mg) and the incubation was continued.

NB in the flask headspace was determined by taking 100-μl samples with a gas-tight syringe and injecting this sample into a gas chromatograph (Hewlett-Packard Model 5890, FID detector) equipped with a 30-m capillary column (DB-608, J&W Scientific, Folsom, CA, USA). Operating conditions were: injector 150°C; oven 150°C; detector, 250°C; nitrogen carrier 6 ml min^{-1}. Under these conditions, NB retention was 2.01 min. Calibration curves were generated by completely evaporating various amounts of NB in closed vials and sampling these vials using a gas-tight syringe. Cell protein was determined according to Bradford [4] and was converted to biomass by use of a factor 2 [11]. Concentrations of nitrite and ammonia were measured spectrophotometrically as described in Standard Methods [1].

For determining the pH optimum for NB removal, washed suspensions of the consortium were suspended in phosphate buffers (0.1 M) adjusted to pH 5.0, 6.0, 7.1, 8.0

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Received 12 September 1996; accepted 17 December 1996

and 9.1. Ten milliliters of these suspensions were placed in 160-ml serum vials closed with Teflon-lined silicone septa and aluminum crimp caps. NB was added at 0.5 μl per vial, and the vials were incubated with shaking (200 rpm) at 28°C for 4 h. NB removal from the headspace was monitored as described for the 1-L culture flasks.

Removal of NB vapors in a trickling air biofilter

For removal of NB vapors from air streams, a trickling air biofilter was used. The details of this apparatus have been published [9]. In brief, microbial biomass, suspended in 0.1 strength fresh mineral medium, was immobilized on perlite (Grace & Co, Cambridge, MA, USA) and packed into a 5×20-cm glass column. Air metered by a needle valve and rotameter and 0.1 strength mineral medium (500 ml) metered by an adjustable peristaltic pump were passed through this column in a unidirectional downflow mode. At the bottom of the column, the mineral medium and the air were separated. The mineral medium was recirculated, while the air was either released or passed through an acid trap (0.04 N H_2SO_4) to remove ammonia. An automatic pH control device for the recirculating mineral medium was available but was not operated for the experiments described. The airstream was partially saturated with NB vapor by letting some of the air pass through a trap containing liquid NB, the other part of the airstream passed through a water trap. Precise metering of the air streams kept NB inlet concentrations at ±10% of the set level. The combined air stream was sampled (100 μl) by means of a gas-tight syringe prior to and after passing through the column, and NB concentrations were determined by gas chromatography. From the rate of the air flow and the concentration change effected by the column, NB removal rates were calculated. They were expressed as g NB removed per m^3 column packing per hour (g m^{-3} h^{-1}). Superficial (linear) air velocities were calculated from the column volume (1.570 ml) and air flow rates. Retention times on the column ranged from 20 to 30 s.

Results and discussion

Utilization of NB by a microbial consortium

A microbial consortium that grew on NB was obtained. From the consortium, four distinctive strains that grew on mineral agar medium under NB vapor were isolated. All strains were Gram-negative rods. Two of the isolates were identified as *Agrobacterium* sp, and the other two were identified as *Sphingomonas* sp and *Achromobacter* sp, respectively.

To determine the yield on NB and the concentrations of ammonia formed, NB was added to a flask at 6 mg daily, and the production of biomass and ammonium in the buffered mineral medium (pH 7.0) was monitored (Figure 1). The cumulative amount of NB added to the flask was compared to the amounts of ammonia produced. Because NB added to the flask was completely removed during incubation, the amount of NB added to the flask was the same as the amount of NB removed. The yield (g dry wt g^{-1} substrate used) on NB was 0.19; 0.30 with NO_2 subtracted. This yield suggests that the consortium converted NB carbon principally to CO_2 and biomass with few, if any,

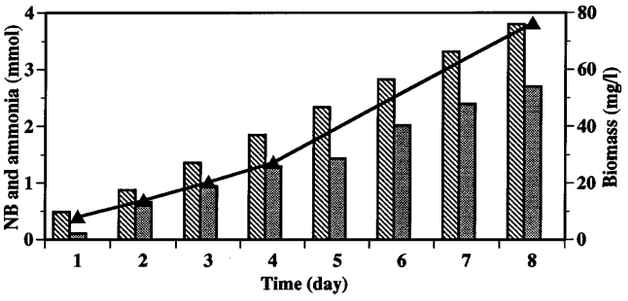

Figure 1 The removal of NB vapor (▨) from the headspace of a flask by a microbial consortium, with the simultaneous production of biomass (▲) and ammonia (▩). The N-free mineral medium was buffered at pH 7.0.

incomplete biodegradation products. Of the NB nitrogen, 73.3% was converted to ammonia-nitrogen. If one assumes that 11.38% of the biomass produced during NB degradation consisted of nitrogen, 16.3% of NB-nitrogen was converted to biomass [10]. During the degradation of NB, nitrite was produced in a very small amount (<40 μg L^{-1}). Therefore, 89.6% of the NB nitrogen was converted to biomass plus ammonia. Ten percent of the nitrogen was missing from the balance.

The pathways of NB utilization by bacteria [12] may involve stepwise reduction of the nitro group to nitroso- and hydroxylamine, followed by a rearrangement to *o*-aminophenol. This is cleaved by an aminophenol dioxygenase to 2-aminomuconic semialdehyde, and the nitrogen is released as ammonium from this metabolite. A more recently described alternative pathway involves a dioxygenase attack, followed by nitrite release from the resulting nitrocatechol. The pathway of NB degradation by the consortium was not investigated, but the release of almost all of the NB-nitrogen as ammonium was a strong indication that the NB degradation pathway followed by the consortium was the same as the one first described by Nishino and Spain [8].

NB removal by the consortium had a pH optimum around pH 7.1, but the optimal range was broad, and 85% or more of the maximum removal was observed between pH 5.0 and pH 9.0 (data not shown). Although the utilization of NB, due to the release of excess ammonium, shifted the pH in the alkaline direction, the broad pH optimum for the consortium allowed us to operate the trickling air biofilter without pH control.

Removal of NB vapor in a trickling air biofilter

The relatively high toxicity of NB to the consortium made it difficult to obtain a dense suspension for immobilization on the perlite packing. This necessitated inoculation and a gradual buildup of the consortium biomass on the filter itself. After inoculation, the filter was operated at low superficial air velocity (3.05 m h^{-1}) at an NB concentration of 26 mg m^{-3}. Under these conditions, NB was removed at the low rate of 0.4 g m^{-3} h^{-1}. During a 4-week period, superficial air velocities and NB concentrations were raised stepwise to 39.8 m h^{-1} and 80 mg m^{-3}, respectively, resulting in an enhanced NB removal rate of 13.1 g m^{-3} h^{-1} at a retention time of 21 s. Recirculation of the liquid

medium was essential for continued biofiltration activity, but pH control had no obvious benefit and was discontinued. Without an inoculum and biomass buildup, an abiotic control column removed NB vapors for a maximum of 2 days. Thereafter, inlet and outlet NB concentrations became identical (data not shown).

Figure 2 shows results from an experiment with the inoculated column, starting after a 4-week conditioning period. During this experiment, water was recycled at 0.2 L h^{-1}. The superficial air velocity was fixed at 34.2 m h^{-1} and the inlet concentration of NB was varied from 100 to 300 mg m^{-3}. During the first 2 weeks of the experiment, NB removal increased and rates up to 70 g m^{-3} h^{-1} were recorded; 50 g m^{-3} h^{-1} was a typical sustainable rate. Under the latter conditions, 80–90% of the NB was removed on the column. Lower emissions were achieved by slightly longer (40 s) residence times (data not shown). In addition to NB removal, the pH of the recycling water and its nitrite and ammonia contents were measured. During the 4-week conditioning period, the pH of the recycling water increased from an initial 7.0 to about 8.7 and remained steady at this value. A very small amount of nitrite was produced and the concentration of ammonia did not increase beyond the steady-state value. This phenomenon could be best explained by the volatilization of dissolved ammonia at the relatively high pH of 8.7. Volatilization of ammonia at high pH is a well-known phenomenon and one of the common ways to remove ammonia from wastewater [3]. Ammonia volatilization was quantified by attaching an acid trap to the exit of the trickling filter. Figure 3 shows that due to the increased pH, ammonia produced during NB degradation was not retained. During the period from day 2 to day 4, almost all of the ammonia produced collected in the acid trap and 98.4% of the NB nitrogen was converted to ammonia nitrogen. This indicates that after the initial conditioning period, there was little or no further increase in biomass and the trickling air biofilter operated essentially in a steady state. Correspondingly, the pressure drop failed to increase measurably during 4 months of operation.

As the consortium could operate efficiently at pH 8.7 and ammonia was volatilized, pH control was unnecessary. The

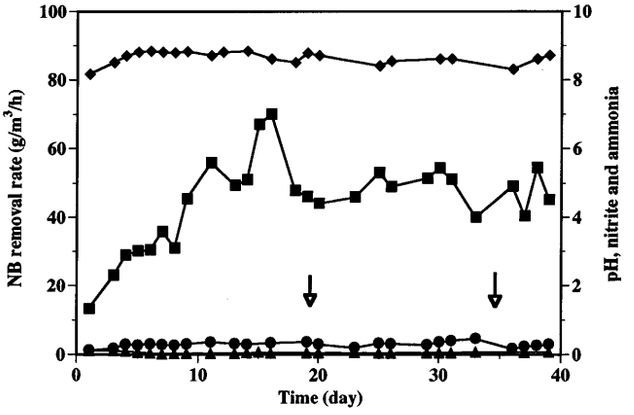

Figure 2 The removal rate of NB vapor in a trickling air biofilter (■), with levels of pH (◆), nitrite (mg L^{-1}) (▲) and ammonia (mg L^{-1}) (●). The arrows indicate the times the recirculating liquid medium was exchanged. The inlet concentrations of NB were set between 100–300 mg m^{-3}.

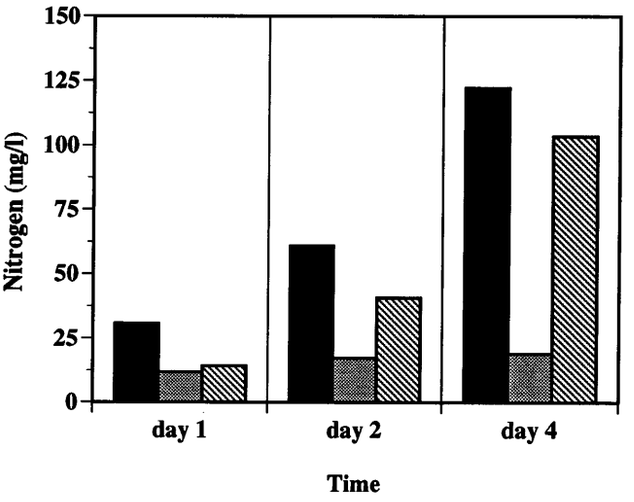

Figure 3 Nitrogen balance during NB removal in a trickling air biofilter. Nitrogen derived from NB removal (■) compared to ammonium present in the recycling medium (▨) and captured as ammonia by the attached acid trap (▧). The latter two balanced the former.

operation of the filter without pH control also avoided the problem of salt accumulation [9]. Nevertheless, replacing the dilute recirculating medium every 2 weeks was beneficial. Whether this removed waste products or added nutrients was not determined. With this minimal maintenance, the filter was operated for over 4 months without any problem, and the results suggest that such filters offer an emission control and bioventing alternative for NB.

Immobilization of the microbial consortium on the perlite was a spontaneous process. The microbial suspension was added to the dry perlite which soaked it up by capillary forces. When the packing was placed in the column, aeration was started immediately but circulation of the liquid was started 12 h later. The liquid phase remained clear and all biomass developed as a biofilm adhering to the perlite packing. The circulating liquid helped to dilute the ammonia produced by the biofilm and facilitated ammonia stripping. In addition, it may have supplied the immobilized biomass with essential nutrients and/or diluted its metabolic waste products.

In conclusion, a trickling air biofilter was shown to remove NB vapors from air streams efficiently and for long periods of time. Scaled-up versions of such devices should help in attaining the goals of the 1990 Amendments to the US Clean Air Act.

Acknowledgements

This New Jersey Agricultural Experiment Station Publication No. D-01122-02-96 was supported by the US Department of Defense, Office of Naval Research and Advanced Research Projects Agency, University Research Initiative Program (Grant N-0014-92-J-1888, R&T a40r41rnri) and State funds.

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