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Enrichment and kinetics of biodegradation of 1-naphthylamine in activated sludge

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Abstract. Sequencing-batch reactors were used to develop an activated sludge enrichment culture capable of degrading 1-naphthylamine (1NA). Approximately 5 months acclimation with salicylic acid (1600 mg l^{-1}) as the primary source of carbon were required to obtain an enrichment culture able to degrade even small quantities of 1NA. After an additional 4 months acclimation, during which the concentration of salicyclic acid was decreased to 50 mg l^{-1}, a culture developed that degraded 1NA concentrations as high as 300 mg l^{-1}. Kinetic determinations showed that 1NA degradation (in the presence of salicylate) followed Michaelis-Menten kinetics with K_m and V_m values of 32.5 ± 2.2 mg l^{-1} and 375 ± 18 ng 1NA mg^{-1} cells h^{-1}, respectively. The same enrichement was able to degrade 1NA when present as the sole source of carbon and energy and to convert approximately 87% to CO_2.

Introduction

1-Naphthylamine (1NA or 1-amino-naphthalene) is a chemical intermediate in the manufacture of azo dyes (coupling agent), herbicides, and a rubber antioxidant (phenyl-alpha-naphthylamine) (Proctor and Hughes 1978; Boeniger 1980; Buckingham 1982). In addition, the compound is an intermediate in the manufacture of rubber-covered cables, pigments, paints, plastics, and toning prints made with cerium salts (Scott 1962; Merck 1989). Other sources of 1NA include wastes from direct coal liquifaction processes (Wilson et al. 1985), and probably all destructive distillation processes used in oil refining and in manufacturing petrochemical products (Staff 1974). The largest potential pollution source of 1NA to the environment is likely to be from the dyestuffs industry where azo compounds comprise approximately 60% of all organic coloring dyes in use today (Boeniger 1980). In 1988, 280 million lbs of dyes were produced in the US (US International Trade Commission 1988). There are approximately 3000 azo dyes currently in use (Holme 1984), and of these, 150 list 1NA as an intermediate in production (Society of Dyers and Colourists 1975). E.I. duPont de Nemours Inc. is the only United States company that produces the chemcial, and their 1972 sales of it and its derivatives were $4.4 million to 560 users (Staff 1974). In 1974 the Occupational Health and Safety Administration (OSHA) regulated 1NA as a carcinogen (US Congress 1974). It is also controlled in the U.K. by the Carcinogenic Substances Regulations of 1967, QM400000 (Buckingham 1982). Approximately 35395 workers were exposed to 1NA in 1972–74 in the United States (Boeniger 1980).

Unsubstituted naphthalene is rapidly degraded aerobically by activated sludge, and the degradation pathway is well known (Gibson 1984). However, degradation of an amino-polyaromatic hydrocarbon may not follow a simple sequence of deamination to naphthalene followed by degradation by the corresponding pathway. We report here biodegradation of 1NA including conversion to CO_2, its percentage removal by non-biological mechanisms (volatilization and sorption), and its kinetics of removal by biodegradation. Mineralization of 1NA has not been reported previously and there was conflicting data in the literature regarding its fate in activated sludge.

Materials and methods

Chemicals and media. Aromatic compounds (except salicylic acid) were obtained from Sigma (St. Louis, Mo., USA) and Aldrich (Milwaukee, Wis., USA). All solvents were HPLC grade and obtained from Fisher Scientific (Pittsburg, Pa., USA). The inorganic mineral salts base used throughout this study consisted of (mg l^{-1}); KH_2PO_4 (200), K_2HPO_4 (125), $(NH_4)_2SO_4$ (100), $CaCl_2 \cdot 2H_2O$ (10.52), $MgCl_2 \cdot 6H_2O$ (16.40), Na_3 citrate (1.765), $FeCl_3$ (0.195), $MnCl_2 \cdot 4H_2O$ (0.0475), $ZnCl_2$ (0.033), $CoCl_2 \cdot 6H_2O$ (0.029), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.021), $CuCl_2 \cdot 6H_2O$ (0.0205), $Na_2B_4O_7 \cdot 10H_2O$ (0.012) in tap water. After 5 months enrichment, we also incorporated the following vitamin solution (mg l^{-1}); pyridoxamine dihydrochloride (0.15), nicotinic acid (0.10), thiamine hydrochloride (0.10), Ca-D(+)-pantothenate (0.05), 4-aminoben-

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zoic acid (0.04), D(+)-biotin (0.01). The concentrated salicylic acid medium consisted of mineral base and vitamins plus 1600 mg l^{-1} salicyclic acid and 160 mg l^{-1} yeast extract. The dilute medium consisted of mineral base and vitamins plus 50 mg l^{-1} salicyclic acid, and 10 mg l^{-1} yeast extract. The beef extract/bacto-peptone medium consisted of mineral base and vitamins plus 100 mg l^{-1} bacto-peptone, and 75 mg l^{-1} beef extract. Yeast extract, beef extract and bacto-peptone were obtained from Difco (Detroit, Mich., USA); salicyclic acid, all inorganic salts and all vitamins except biotin and pyridoxamine were obtained from Fisher Scientific. Biotin and pyridoxamine were obtained from Sigma.

Analytical methods. Cell growth was monitored by dry weight determination (method 2540 E; total non-filterable volatile residue at 550° C) (APHA 1989); dissolved oxygen was monitored with a Yellow Springs Instrument Co. model 58 dissolved oxygen (DO) meter with model 5720A probe (Yellow Springs, Ohio, USA). Aromatic compounds were analyzed by HPLC and/or gas chromatography (GC). The HPLC analysis was performed with a modified Dionex Corporation (Sunnyvale, Calif., USA) 4000i gradient ion-chromatography system equipped with either a Dynamax-Microsorb C18 or a Drynamax-Macro C8 column from Rainin Instrument Co. (Woburn, Mass., USA), a Linear Instrument Co. (Reno, Nev., USA) UV-200 Spectrophotometric detector, and a Hewlett Packard (Avondale, Pa., USA) 3396A integrator; the column temperature was 25° C, the mobile phase was 70% acetonitrile, 30% water at either 1.0 (C18 column) or 2.0 (C8 column) ml min^{-1} flow rate, the sample size was 100 µl, and detection was at 244 nm. The ion chromatograph was modified to operate as an HPLC with the addition of a Valco C10U air-actuated sample loop injection valve (Alltech, Deerfield, Ill., USA).

The GC analysis was performed on a Varian (Palo Alto, Calif., USA) Vista 6000 GC equipped with a 30-m X 0.25-mm RSL-200 Heliflex bonded polydiphenyldimethylsiloxane 1.0-µm film capillary column (Alltech), a flame ionization detector, and a Shimadzu Corporation (Kyoto, Japan) C-R3A integrator. The injection technique was splitless with septum sweep (30 ml min^{-1}) flow injected automatically at 30 s after injection of 2-µl samples. The following operating conditions were used: injector temperature, 280° C; column temperature, 55° C (hold 2 min) to 190° C at 20° C min^{-1} (no hold) then to 280° C at 8° C min^{-1} with a final hold of 3 min; detector temperature, 325° C; helium carrier gas flow velocity, 30 cm s^{-1} (0.88 ml min^{-1} flow rate). Under these GC conditions, 1NA had a retention time of 16.8 min and a detection limit of 1.0 ng or 0.007 nmol. For the same conditions, unsubstituted naphthalene eluted at 12.4 min. Under these HPLC conditions, the detection limit for naphthalene was higher at 50.0 ng or 0.350 nmol. Gas samples were analyzed for CO_2 by GC. A Hewlett Packard 5890A GC equipped with a 3-m X 3-mm stainless steel column packed with activated carbon, a thermal conductivity detector (TCD), and Hewlett Packard 3392A integrator. The following operating conditions were used: injector temperature, 60° C; detector temperature, 60° C; column temperature, 80° C; helium carrier gas flow rate, 60 ml min^{-1}; TCD helium reference gas flow rate, 75 ml min^{-1}; sample size, 1 ml. Under these GC conditions, CO_2 had a retention time of 1.09 min and a detection limit of less than 300 ppm (air).

Preparation of samples for analysis. Bulk liquid samples with cell pellets (5, 14, or 100 ml) were collected and centrifuged at 2575 *g* for 5 min (5 and 14 ml sample sizes) or vacuum filtered through 1.5-µm Whatman 934AH glass microfiber filters (100 ml sample size). Four or 10 ml of the centrifuged samples or 100 ml of the filtered samples were extracted using solid-phase extraction with a Vac-Elute (Analytichem, Harbor City, Calif., USA) ten-place vacuum manifold at 25-in Hg vacuum onto 100 mg C18 bonded silica sorbent cartridges (1 ml). The adsorbed compounds were then eluted with 2 ml methanol into 3.7-ml amber serum vials and sealed with screw caps and teflon-lined neoprene septa until analysis. Most samples were analyzed by both GC and HPLC within 8 h; others were stored at 4° C until analysis. The disposable extraction columns were first conditioned with 1 ml methanol for 5 min, then washed with 2 ml deionized water. The sample (4, 10, or 100 ml) was then eluted through the column at 10–30 ml min^{-1}, the column was washed again with 2 ml deionized water and dried under vacuum for 10 min. Finally, the sample was eluted twice with 1.0 ml methanol, with a 2-min contact time for each 1 ml of methanol before elution. At the highest sample volume (100 ml), this gave detection limits of 10.0 µg l^{-1} for 1NA and 100 µg l^{-1} for naphthalene.

Efficiency of recovery in the extraction procedure was a function of concentration, with lower concentrations giving lower recoveries. For this reason, standards were formulated in four separate concentration ranges for the extraction procedure, GC, and HPLC. Recovery efficiencies for the extraction procedure varied by less than 5% within each concentration range but were different for each range (between 80 and 100%). The use of four separate ranges contributed to correlation coefficients (r squared) in excess of 0.90 for all standard curves. For each of the GC and HPLC standard curves, a 95% central confidence interval equation for the regression line was determined and used to calculate error bars whenever replicate samples were not available. Cell pellets were extracted with toluene or methanol. Ten millilitres of the chosen solvent was added to the previously centrifuged and decanted pellets in a centrifuge tube, which was stoppered and shaken vigorously by hand for 1 min. Following mixing, the cells were centrifuged for 5 min and the supernatant analyzed by GC and/or HPLC.

Activated sludge enrichment cultures. Activated sludge was collected from the Hyperion waste-water treatment plant in El Segundo, Calif., USA, and a west-coast petroleum oil refinery. Hyperion is a large waste-water treatment plant which receives mostly municipal sewage and some industrial wastes. Naphthalene, but not 1NA, was reportedly detected in the Hyperion influent several times per year at concentrations of 2–20 µg l^{-1}. The oil refinery is a large (300000 barrel day^{-1}), diversified refinery with a wide variety of operations, including catalytic cracking, coking, and reforming. The possibility of prior exposure of the refinery activated sludge to 1NA is high.

A 50/50 mixture of activated sludge from these two sites was used as inocula for one 5-l and 14-l sequencing batch reactor (SBR) (initial solids dry weight of approximately 2000 mg l^{-1}). In general, SBRs operate on a periodic cycle made up of four discrete time periods (Irvine and Ketchum 1989). These consist of (1) an aeration or reaction period, followed by (2) a settling period when aeration or mixing is stopped and gravity separation of supernatant and solids occurs. The next step (3) is a drain period to withdraw supernatant and/or cells. Finally (4), fresh media is added during the fill period, and the cycle repeats in the same sequence. The SBRs used here were all operated on a 24-h cycle with a 22-h aeration time, 1-h setting time, and 0.5-h drain and fill times.

The SBRs were constructed of high density polyethylene plastic and blackened with dark window tinting material. They were operated at 20–25° C, pH 6.5–8.0, and sparged with compressed air at the rate of 5 or 15 l min^{-1} (5-l and 14-l SBRs, respectively) using spherical fine-pore aeration stones. The DO concentration varied between 4.0 and 6.0 mg l^{-1}. The solids retention time (SRT) was controlled by removal of enough bulk liquid from the SBRs just prior to the end of the aeration period to provide a nominal solids retention time of 14 days. The assumptions used in the operation of these SBRs are the same as those used in actual practice; namely that the setting period is of sufficient duration to allow most of the floc-forming bacteria to settle by gravity into a thick sludge blanket on the bottom of the tank, and only settleable organisms are used since all suspended cells are wasted during the drain cycle. In the general case the drained volume would be a set value; however, we removed as much of the supernatant as possible (approx. 4 and 10 l from the 5-l and 14-l SBRs, respectively).

During the first 5 months of acclimation, the 5-l SBR was maintained on beef extract/bacto-peptone medium, and the 14-l

SBR received concentrated salicylic acid medium. Both SBRs also received 3 mg 1NA l^{-1} daily for 5 months prior to the detection of significant depletion of 1NA by GC and HPLC analysis. Dry cell weight and supernatant 1NA concentrations were monitored weekly (samples were taken at the end of the 22-h aeration period). Good growth was observed and cell mass remained between 3–6 g l^{-1} (upper and lower limits for the entire period) during this phase while the SRT was maintained at 14 days. After 5 months, the 14-l SBR culture was able to deplete 3 mg l^{-1} of 1NA during the 22-h aeration period. The daily 1NA spike was then increased to 10 mg l^{-1} for a period of 2 months, after which time the medium was changed from concentrated to dilute salicylic acid medium. Over the next 2 months, the 1NA concentration was gradually increased from 10 to 50 mg l^{-1}. Supernatant 1NA concentrations were measured daily at the end of the aeration period. If the 1NA concentration was below the detection limit on two consecutive days, the daily 1NA spike was increased by 10 mg l^{-1}. If there remained a detectable 1NA concentration, the SBR cycle was repeated without an increase in magnitude of the 1NA spike. During this period (6th and 7th months), growth was not as vigorous and dry cell weight decreased nearly continuously to approximately 1.1 g l^{-1}. The 5-l SBR (maintained on beef-extract/bacto-peptone medium) did not have the same degradative abilities as the dilute salicylic acid enrichment and was discarded at the end of the 7th month.

After the 7th month, some of the cells from the 14-l SBR were transferred to a new 5-l reactor (2.5 l from the 14-l reactor plus 2.5 l of dilute salicylic acid medium). Dilute salicylic acid medium was used in all subsequent experiments. The transferred culture was not operated in the SBR mode. Instead, it was inoculated with 1NA and fresh media and monitored every 12 or 24 h for 1NA depletion and not reinoculated with fresh media or 1NA until the supernatant 1NA concentration was below the detection limit. Once 1NA was no longer detectable, aeration was stopped and cells allowed to settle for 1 h. This was followed by removal of supernatant, addition of fresh media, and reinoculation with 1NA (no cells were intentionally wasted). This is called the fed-batch mode operation. This fed-batch reactor was operated for 43 days to assess the degradative abilities of the 14-l SBR enrichment culture and not to develop a different enrichment culture. While experiments were being conducted using the new 5-l fed-batch reactor, the 14-l SBR enrichment was maintained on dilute salicylic acid medium and 1NA concentrations that were gradually increased from 50 to 150 mg l^{-1}. This culture is still maintained on 150 mg 1NA l^{-1} and has been used in enricher-reactor bioaugmentation experiments described elsewhere (Babcock et al. 1991).

Results

Fed-batch degradation of 1NA in the presence of salicylate

The 5-l fed-batch reactor was maintained on dilute salicylic acid medium and initially spiked with 50 mg 1NA l^{-1}, followed by two cycles each of 100, 150, 200, and 300 mg 1NA l^{-1} (see Fig. 1 and Tables 1 and 2). This culture was incubated at 20–25° C, pH 6.5–7.5, an air sparge rate of 5 l min^{-1}, and solids were not wasted. An identical reactor with media, 1NA, the same air sparge rate, and no biomass was operated as a volatilization control. Overall losses from the control reactor during each incubation cycle varied between 8 and 15% irrespective of the initial concentration (data not shown). Supernatant and cell-adsorbed 1NA concentrations are shown in Fig. 1 (error bars represent 95% confidence intervals of GC standard curves), and cell dry weights are given in Table 1 (single determinations, no replicates).

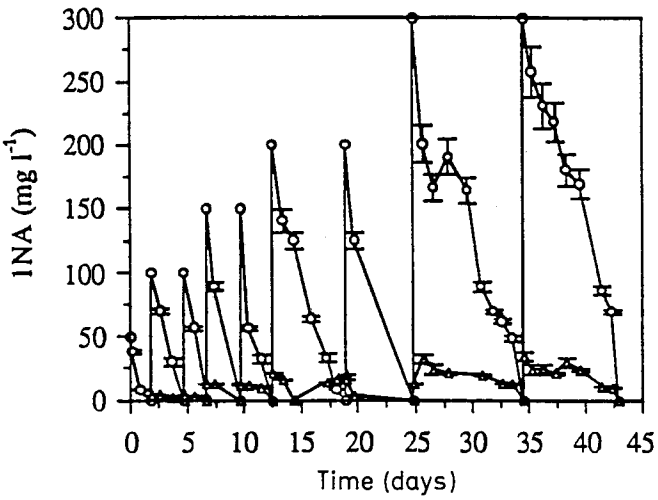

Fig. 1. Fed-batch degradation of 1-naphthylamine (1NA) in dilute salicylic acid medium: supernatant (O) and cell-adsorbed (Δ) 1NA concentrations

Table 1. Cell dry weight during fed-batch experiments

Day	Dry weight (mg/l)
0	580
5	796
10	1282
18	1630
25	1658
35	1960
43	2432

Table 2. Total time for degradation of supernatant 1-naphthylamine to below detectable levels in fed-batch experiments

Initial concentration (mg/l)	Time (h)	
	First incubation	Second incubation
50	—[a]	44
100	68	48
150	72	67
200	155	140
300	234	203

[a] Data not available

The time duration for degradation of 1NA to the detection limit (in this case, 100 μg l^{-1}) was always shorter during the second incubation period (Table 2). During these experiments, cell dry weight increased from an initial value of 580 mg l^{-1} to 2432 mg l^{-1} after 43 days (no solids were wasted, see Table 1).

The average rate of 1NA degradation per unit biomass (initial 1NA mass added/time to reach detection limit x initial biomass dry weight) for the second incubation period at each concentration for the fed-batch experiments is given in Fig. 2 (no replicates). The maximum rate was approximately 0.2 mg 1NA mg^{-1} cells

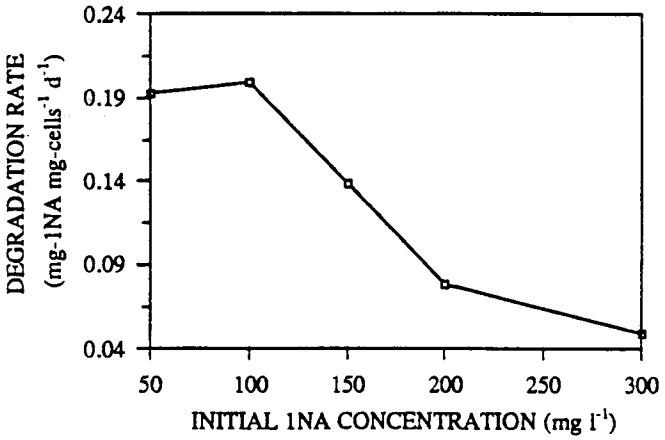

Fig. 2. Average degradation rate of 1NA per unit biomass as a function of initial concentration. Rates shown are (initial mass of 1NA)/(overall degradation time × initial biomass dry weight) during the second incubation period as shown in Fig. 1

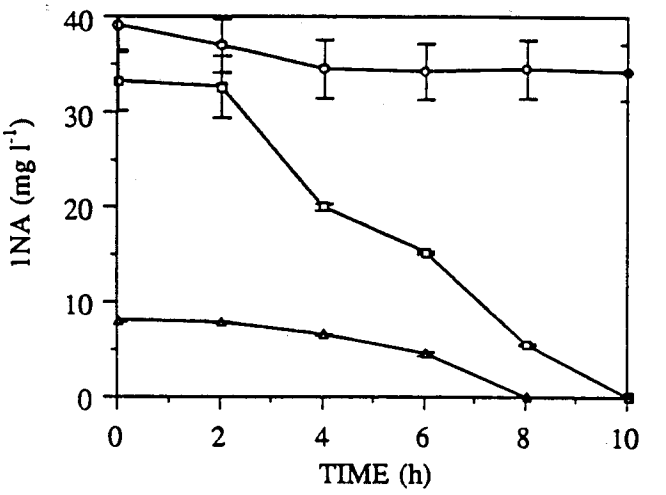

Fig. 3. Degradation of approximately 50 mg l^{-1} of 1NA in a 5-l sequencing batch reactor: supernatant (□) and cell-adsorbed (△) 1NA concentrations, active reactor; supernatant 1NA concentration, control reactor (○)

day^{-1} and occurred for 100 mg 1NA l^{-1} initial concentration. At initial 1NA concentrations greater than 100 mg l^{-1}, the average degradation rate was less than the maximum value, which may indicate some type of toxic effect.

SBR degradation of 1NA in the presence of salicylate

Following the second 300 mg l^{-1} spike, the fed-batch mode was discontinued and the SBR mode was reinstated in the 5-l reactor using the same periodic cycle used previously. The 5-l SBR then received dilute salicylic acid media and 50 mg 1NA l^{-1} for several days, after which its ability to degrade 1NA was re-examined. Figure 3 shows the characteristic pattern of 1NA degradation during the daily SBR aeration cycle. The initial 1NA concentration of approximately 50 mg l^{-1} (adsorbed + supernatant) was injected at the start of the aeration cycle. Supernatant and cell concentrations were monitored in the SBR and a no-cell volatilization-control reactor (error bars represent 95% confidence intervals of GC standard curves). In Fig. 3, the zero time samples taken shortly after spiking with 1NA show significant adsorption. Adsorption of this hydrophobic compound was apparently very rapid. Overall removal of 1NA from the volatilization control was about 5 mg l^{-1} or 12.5%. The total time for degradation of supernatant 1NA to the detection limit in the active SBR was 10 h, which is less than half the duration of the SBR aeration cycle.

Kinetics of 1NA degradation in the presence of salicylate

The 1NA degradation kinetics for the SBR culture were then estimated. We were interested in degradation kinetics in the presence of dilute salicylic acid medium, since the culture was developed for use in bioaugmentation experiments during which salicylate was always present as a growth substrate (Babcock et al. 1991). Four hundred milligrams of washed cells (from the 5-l SBR) were resuspended in 200 ml of dilute salicylic acid medium (initial cell concentration, 2000 mg l^{-1}) in 500-ml erlenmeyer flasks. Flasks were covered with aluminium foil and placed in an enclosed orbital shaker table at 27° C and 200 rpm. Triplicate active flasks and uninoculated controls were incubated with each of the following initial 1NA concentrations: 1, 5, 10, 50, 100, 200, 300, and 500 mg l^{-1}. Degradation of 1NA was monitored by taking 10-ml samples at appropriate intervals followed by extraction and GC or HPLC analysis. Losses from controls were negligible. Initial degradation rates were determined from these data and used to estimate kinetic parameters.

Kinetic parameters were estimated by transforming the data via Lineweaver-Burke, Hanes-Woolf, and Eadie-Hofstee plots. Good agreement was found among the three methods. Toxicity as indicated by a deviation from Michaelis-Menten kinetics occurred for the higher concentrations (200, 300, and 500 mg 1NA l^{-1}) and thus only the lower concentrations were used for determination of kinetic constants. The initial degradation rates were measured and plotted by the three methods and linear curves were obtained indicating Michaelis-Menten kinetics. The values of K_m and V_m were estimated to be 32.5 ± 2.2 mg l^{-1} and 375 ± 18 ng 1NA mg^{-1} cells h^{-1} (227 ± 15 μM and 2.62 ± 0.13 nmol 1NA mg^{-1} cells h^{-1}) respectively. The reported values are averages ± standard deviations of the values obtained from the three methods.

Degradation of 1NA as the sole source of carbon

To determine if the biodegradation of 1NA required cometabolic (Horvath 1972) activity in our SBR enrichment culture, we inoculated cultures with 1NA and

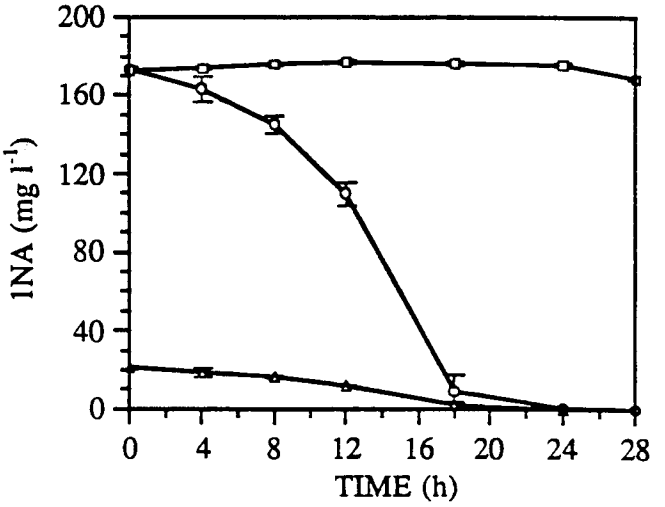

Fig. 4. Shake-flask degradation of 175 mg 1NA l^{-1} as the sole source of carbon at 27° C and 200 rpm: supernatant (○) and cell-adsorbed (△) 1NA concentrations, active reactor; supernatant 1NA concentration, control reactor (□)

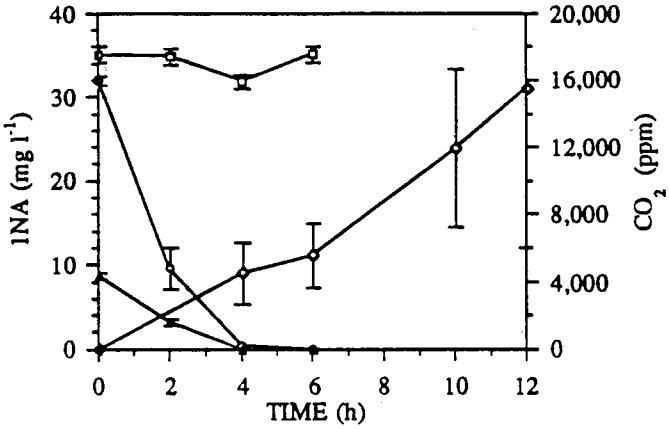

Fig. 5. Mineralization of 35 mg 1NA l^{-1} as the sole source of carbon at 27° C and 200 rpm: supernatant (○) and cell-adsorbed (△) 1NA concentrations, active vials; supernatant 1NA concentration, volatilization control vial (□); amount of net CO_2 produced in active vials (◇)

without any other added carbon source. The media contained inorganic salts base without citrate. Triplicate erlenmeyer flasks (500 ml) were inoculated with 400 mg washed cells in 200 ml medium (initial cell cocnentration, 2000 mg l^{-1}), spiked with approximately 175 mg 1NA l^{-1}, covered with aluminium foil and incubated at 27° C in an enclosed orbital shaker table at 200 rpm (see Fig. 4). Washed cells were prepared by centrifuging the pellets for 5 min and decanting the supernatant, then resuspending the pellets in deionized water and repeating the procedure again, twice. A volatilization control with media, 1NA and no cells was also incubated. Cell-adsorbed 1NA was quantified by extracting the cell pellets with methanol. Figure 4 shows that the enrichment culture was able to transform 1NA without the presence of other carbon sources and at rates similar to those observed in the presence of salicylate and yeast extract. Thus, co-oxidation is not necessary for degradation of 1NA by the present enrichment culture. Error bars represent the standard deviation of triplicate flasks for the supernatant and cell-adsorbed 1NA data. Error bars for the control flask are 95% confidence intervals for the HPLC standard curves.

Mineralization of 1NA to CO_2

To determine whether or not 1NA was degraded to CO_2 (mineralized), cells were exposed to 1NA as the sole source of carbon in sealed vials and the headspace gas was monitored for production of excess CO_2 (see Fig. 5). Using 125-ml serum vials, 60 mg washed cells was resuspended in 30 ml inorganic salts base without citrate (initial cell concentration, 2000 mg l^{-1}). Vials were inoculated with 1NA (except the endogenous control), sealed with Teflon-lined rubber septa and aluminium crimped tops, and placed in an enclosed orbital shaker table at 27° C and 200 rpm. There were four vials; duplicate active vials with cells and approximately 35 mg 1NA l^{-1}; an endogenous control containing cells and media, but no 1NA; and a volatilization control with 1NA and no cells.

Figure 5 shows the degradation of supernatant and cell-adsorbed 1NA in the active vials, and formation of CO_2 in excess of that formed in the endogenous control (CO_2 concentration measured in endogenous control was subtracted). Losses from the uninoculated volatilization control were minimal. Cell pellets were extracted with methanol. Figure 5 shows that more CO_2 was evolved from sealed sample vials containing 1NA than from an identically prepared vial without 1NA (uninoculated). Error bars represent standard deviations of data from duplicate vials for supernatant and cell-adsorbed 1NA as well as CO_2 produced. Error bars for the control vial are 95% confidence intervals for the HPLC standard curves. Although the error bars are quite large for the 12-h CO_2 data, the upward trend is conserved. The 12-h net CO_2 concentration of 15600 ppm corresponds to approximately 0.76 g carbon, and approximately 0.87 g carbon in the form of 1NA was added to the serum vials. Thus, approximately 87% of the 1NA carbon added could be accounted for as CO_2 in 12 h.

Discussion

Because 1NA is inhibitory to many microorganisms, its biodegradation by activated sludge was uncertain. 1NA significantly inhibited nitrification by a mixed population of *Nitrobacter* and *Nitrosomonas* species isolated from activated sludge; a concentration of 15 mg l^{-1} gave a 50% inhibition (Hockenbury and Grady 1977). 1NA was also highly toxic to an axenic culture of the alga, *Selenastrum capricornutum,* even at low concentrations (1% of saturation) (Giddings 1979), and was mutagenic using the *Salmonella*/microsome mutagenicity test (McCann et al. 1975).

1NA as sole carbon source (200 mg l^{-1}) was not degraded after 20 days incubation in activated sludge regardless of previous exposure to 1NA as a secondary substrate (Pitter 1976). 1NA was inhibitory to oxygen uptake of activated sludge as measured by Warburg respirometry at 20 mg l^{-1}, but 82% of the 1NA added disappeared within 6 h (Baird et al. 1977). Apparently, in autoclaved control cultures, less than 3% loss was found, but no data on 1NA mineralization were included. An aniline-acclimated activated sludge fed 500 mg l^{-1} of 1NA exhibited some oxidation, as measured by incubation of Warburg vessels for 192 h (Malaney 1960); however, no quantification of any actual decrease in 1NA was reported so comparison with the present results is difficult. Furthermore, our results do not agree with those of Baird et al. (1977), who reported significant removal of 1NA by unacclimated activated sludge. Our findings showed that 1NA was initially resistant to degradation, and only after several months of enrichment was the culture able to degrade significant quantities of 1NA and to mineralize 1NA to CO_2.

When added as a substrate, naphthalene was degraded rapidly by the present culture. However, in the experiments reported here, naphthalene was never detected as a 1NA degradation product in supernatant or cell-extraction samples. This suggested that either deamination occurred and the resulting naphthalene was degraded too rapidly to detect or that degradation followed a different pathway from that via naphthalene. However, the degradation pathway for 1NA cannot be determined from the experiments reported here. Since the enrichment culture is a consortium of organisms, degradation intermediates were presumably metabolized by the mixed population present. We have isolated organisms from the enrichment culture capable of 1NA degradation and are examining the degradation pathways in these isolates. Of these isolates, one requires the presence of pyruvate to degrade 1NA and another is able to deplete and grow on 1NA without any other carbon sources (neither is able to degrade naphthalene).

Although, 1NA can be degraded when present as the sole carbon source (Figs. 4 and 5), in our reactors, salicyclic acid is always added. The rationale for using salicylic acid is threefold. First, salicylate is a known intermediate in the degradation pathway for naphthalene and may be a co-ordinate inducer of enzymes capable of degrading 1NA (Gibson 1984). Second, salicylate is a relatively inexpensive carbon source with which to grow large numbers of cells for use in bioaugmentation experiments, as described elsewhere (Babcock et al. 1991). Third, 1NA cannot be added as the sole carbon and energy source because of its toxicity (to the enrichment culture) at substrate level concentrations.

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