Effect of Desorption Rate on the Biodegradation of *n*-Alkylamines Bound to Clay

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The resistance to microbial attack of an homologous series of *n*-alkylamines bound to clay was correlated with their molecular weights. Bentonite–*n*-[1-^{14}C]decylamine was prepared to test whether the rate of microbial degradation of the sorbed amine was limited by the rate of its desorption into the surrounding liquid containing bacteria utilizing the chemical. The desorption rate was estimated using a continuous flow diafiltration cell. The rate of biodegradation of bentonite–*n*-[1-^{14}C]decylamine, measured as $^{14}CO_2$ evolution, was found to depend on inoculum size, and it probably exceeded the desorption rate at high bacterial densities. Thus, microorganisms may facilitate removal of the sorbed substrate from the clay.

The sorption of certain organic chemicals to clay and colloidal soil organic matter strongly influences their biodegradation by microorganisms. Both stimulation and retardation of biodegradation of proteins bound to clay have been observed. Protection of the substrate from decomposition is often associated with sorption within the inner lattice of expanding lattice clays, but even in this location, molecules are not necessarily inaccessible to microorganisms or their extracellular enzymes (Estermann et al., 1959). Data obtained for smaller organic molecules sorbed to colloidal surfaces, and presumably decomposed by intracellular enzymes, are limited chiefly to pesticides and some of their metabolites. For example, the cationic herbicides diquat and paraquat appear to be completely protected from degradation when adsorbed to montmorillonite (Weber and Coble, 1968; Burns and Audus, 1970).

Because thousands of industrial and agricultural chemicals are being discharged onto soil and into waterways containing particulate matter, it is important to understand the mechanisms controlling the frequently slow degradation of sorbed chemicals that are normally biodegradable. To this end, studies were conducted of a relatively simple model system made up of an homologous series of *n*-alkylamines that were complexed with bentonite clay and suspended in an inorganic salts solution. These amines, or more specifically their salts, are bound to clay by a cation-exchange mechanism investigated by Cowan and White (1958), who found that the ease of adsorption and strength of binding between the alkylamine salts and clay increased with molecular weight of the amine. The present investigation was designed to determine the effect of molecular weight on microbial utilization of sorbed amines and to establish whether the rate of biodegradation of a sorbed substrate is equal to its rate of desorption.

EXPERIMENTAL SECTION

Chemicals. *n*-Hexylamine and di-*n*-butylamine were obtained from Eastman Kodak Co. (Rochester, NY), *n*-heptylamine and *n*-octylamine from Sigma Chemical Co. (St. Louis, MO), and *n*-decylamine from Pfaltz and Bauer (Flushing, NY). They were used without further purification. *n*-[1-^{14}C]Decylamine hydrochloride, sp act. 1.1 mCi/mmol and 98% radiochemical purity, was obtained from California Bionuclear Corp., Sun Valley, CA. D-[^{14}C(U)]Glucose, sp act. 340 mCi/mmol and 97% radiochemical purity, was obtained from New England Nuclear (Boston, MA), and it was diluted by about 400:1 with unlabeled D-glucose. The inorganic salts solution contained 1.6 g of K_2HPO_4, 0.40 g of KH_2PO_4, 0.50 g of NH_4NO_3, 0.20 g of $MgSO_4 \cdot 7H_2O$, 25 mg of $CaCl_2 \cdot 2H_2O$, 1.4 mg of $FeCl_3$, and 1000 mL of distilled water. The pH of this solution was 7.2.

Complex Formation. Montmorillonite (bentonite) from Clay Spur, WY, was provided by Wards Natural Science Establishment, Rochester, NY. Size fractions of particles less than 1 μm in diameter were separated by gravity settling according to the Stokes equation. When suspended in deionized water, the clay preparation had a cation-exchange capacity of 4.2 *me*/100 g of suspension with 2.2% solids by weight, corresponding to a cation-exchange capacity of 191 *me*/100 g of the montmorillonite itself. Complexes of each of the alkylamines with the bentonite were made by stirring 20 g (0.84 *me*) of the clay suspension for 24 h with 0.1 *me* of the amine, which had been previously dissolved at concentrations of 300–500 μg/mL in distilled water and adjusted to neutrality with 1 N HCl. The clay complexes were collected by centrifugation at 12000*g* and washed twice with distilled water. The final supernatant solution separated from each bentonite–amine complex and all of the washings were analyzed by gas chromatography as described below. In each instance, the amount of uncomplexed amine was below the limit of detection, i.e., 5 ng/μL or less.

A bentonite–*n*-[1-^{14}C]decylamine complex was made in a similar fashion. Five grams of a suspension of bentonite particles of <1 μm (0.21 *me*) was stirred with 16 mL of an aqueous *n*-[1-^{14}C]decylamine hydrochloride solution containing 2.88×10^6 cpm/mL and 0.025 *me* of the amine. The separated complex was washed five times with deionized water, and each washing was tested for the appearance of AgCl when a $AgNO_3$ crystal was added. Only in the first wash solution did the white precipitate appear. A 1-mL portion of each washing solution and of the final supernatant solution separated from the complex was added to 10 mL of Aquasol (New England Nuclear, Boston, MA) for liquid scintillation counting (Beckman LS-100C liquid scintillation system). About 6% or less of the *n*-[1-^{14}C]decylamine was lost from the complex during the separation and washing procedure, probably because the finest particles were not precipitated at 12000*g*.

Biodegradation. The rate and extent of degradation of each of the bentonite–*n*-alkylamine complexes, representing 60–150 μg/mL of amine suspended in the salts solution, were compared with the decomposition of the corresponding amine free in the salts solution also at 60–150 μg/mL. The buffered solutions of the amines were readjusted as needed with 1 N HCl to a pH of 7.2. The inoculum was a mixed culture of bacteria grown on the individual amines as sole carbon source to give an initial level of about 10^6 cells/mL. The original source of the organisms was Lima loam. The solutions and suspensions were incubated at 30 °C in the dark on a rotary shaker operating at 90 rpm. At selected time periods, samples

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0021-8561/79/1427-0410$01.00/0

were removed and centrifuged at 12000g and 5 °C to remove cells and separate the complex. The clay complex was dissolved in 5 N HF to release bound amines, and the liquid was neutralized with 5 N NaOH and centrifuged. All solutions and clay extracts were then analyzed with a Perkin-Elmer 3920B gas chromatograph equipped with a 60 × 0.30 cm glass column packed with Tenax GC (60–80 mesh, Applied Science Laboratories, State College, PA) and a flame ionization detector. Analyses were performed isothermally for the individual amines: n-hexyl-, 160 °C; n-heptyl-, 175 °C; n-octyl-, 185 °C; di-n-butyl-, 170 °C; and n-decyl-, 205 °C. Helium was used as carrier gas at 20 mL/min. The injector and manifold temperatures were both 230 °C. The limit of detectability for these polar amines was about 5 ng/μL. Peak areas of individual amines were used for quantification. Although duplicate samples were not taken at the various time periods, entire experiments were repeated, and the results were essentially the same in each instance.

The degradation of bentonite–n-[1-^{14}C]decylamine was monitored by measuring the production of $^{14}CO_2$ with time. The complex suspended in the salts solution amounted to 12 μg/mL of n-[1-^{14}C]decylamine, and the percentage of solids was about 0.014%. The Hobbie and Crawford (1969) method was used for trapping and measuring evolved $^{14}CO_2$. Individual 25-mL flasks containing the bacteria and identical amounts of complex suspended in the salts solution were sacrificed periodically for measurements. The mixed bacterial culture used as inoculum contained 2 × 10^{10}, 6 × 10^9, and 2 × 10^8 cells/mL, and the flasks were incubated at 22 °C on a reciprocating shaker operating at 130 strokes/min. A control solution of 12 μg/mL of n-[1-^{14}C]decylamine hydrochloride in the salts solution was inoculated to give an initial cell density of 2 × 10^8 cells/mL. To prepare the inoculum, the bacteria were grown on 300 μg/mL of n-decylamine for about 24 h, and the cells were collected by centrifugation, washed, and concentrated about 300-fold to achieve the highest cell densities used. The cell density was determined by counting on nutrient agar.

Isolation of Pure Cultures Growing on Decylamine. From the mixed bacterial culture growing on n-decylamine, two pure cultures were isolated. The isolates, which were Gram-negative, motile rods, were grown in the salts solution supplemented with 300 μg of n-decylamine/mL.

Desorption Rate. A modified Amicon Model 12 magnetically stirred 10-mL ultrafiltration cell (Amicon Corp., Lexington, MA) equipped with a Nuclepore N010 0.1-μm polycarbonate membrane (Nuclepore Corp., Pleasanton, CA) and an 800-mL reservoir was used for continuous flow dialysis and determination of the rate of desorption of n-[1-^{14}C]decylamine from its bentonite complex into the surrounding salts solution. The cell was modified by removing the polyethylene porous membrane support disk and substituting a stainless steel support screen plus a Teflon O-ring (Millipore Corp., Bedford, MA). Bentonite–n-[1-^{14}C]decylamine was suspended in 4.6 mL of the salts solution with the $FeCl_3$ omitted to prevent deposition of a gelatinous hydrous ferric oxide precipitate on the membrane. This suspension corresponded to a concentration of 12 μg/mL of the labeled amine and contained about 0.014% solids. The salts solution (minus $FeCl_3$) filled the reservoir and flowed through the cell at a rate of 4.3 mL/min under 18 psig N_2. This was the highest flow rate obtained with this suspension, even at higher pressure. However, dilution of the suspension plus pressures up to 40 psig N_2 allowed for flow rates up to 9 mL/min. On the other hand, decreasing the pressure resulted in flow rates down to 1 mL/min. A series of diafiltrations was performed with the flow rates varying from 1–9 mL/min. In each diafiltration, a fraction collector captured the eluate in sequential tubes, 500 drops or about 16 mL per tube or, at high flow rates, the tubes were advanced every 1–2 min. Diafiltrations were performed at 22 °C and were terminated when 500–700 mL of eluate was collected. A 1-mL portion was removed from each tube for liquid scintillation counting in 10 mL of Aquasol. Aliquots of selected tubes were centrifuged for 3 h at 81000g and 4 °C to estimate possible interference from very fine particles (<0.1 μm) of bentonite–n-[1-^{14}C]decylamine, but no significant difference in cpm was found when the solutions were counted before and after ultracentrifugation.

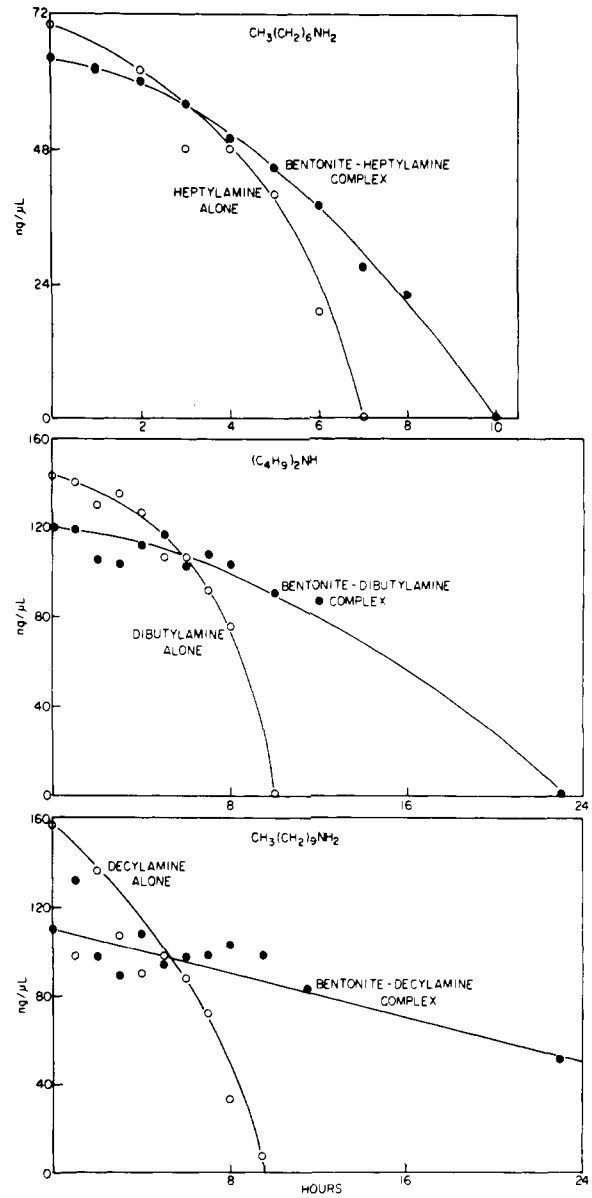

Figure 1. Degradation of bentonite–n-alkylamines and n-alkylamines free in an inorganic salts solution.

The counting efficiency for all liquid scintillation data was not determined, but channels ratios measurements were all within 2 to 3%.

RESULTS AND DISCUSSION

For the biodegradation of the bentonite–n-alkylamine complexes studied, an increased resistance to microbial attack was observed as the molecular weight of the ad-

sorbed amine increased. Figure 1 shows this trend with data plotted for *n*-heptyl-, di-*n*-butyl-, and *n*-decylamine, but tests were also conducted of *n*-hexyl- and *n*-octylamines. All of the adsorbed amines studied appear to have been completely degraded in 1 or 2 days, i.e., the concentrations in the extracts fell below the limit of detectability. The sorbed hexylamine was decomposed in approximately the same time period (9 h) as *n*-hexylamine free in solution. The complexed heptylamine was degraded somewhat more slowly (only 53% lost in 7 h) than free *n*-heptylamine, which had disappeared in 7 h. Only 27% of the complexed octylamine was degraded in 12 h, while all of the free *n*-octylamine had apparently been decomposed at that time. Finally, only 20% or less of the bentonite–*n*-decylamine had disappeared by the time all of the free *n*-decylamine was degraded. The secondary amine, di-*n*-butylamine, behaved in a similar fashion to the primary amine having the same molecular weight, *n*-octylamine. Only 25% of the dibutylamine sorbed to the clay was decomposed in 10 h, whereas all of the free di-*n*-butylamine was decomposed at that time. Since secondary amines are precursors for the formation of nitrosamines, especially in the presence of adsorptive surfaces such as colloidal soil organic matter (Mills and Alexander, 1976), their persistence in these circumstances is noteworthy.

Cowan and White (1958), who studied the mechanism of cation-exchange reactions between montmorillonite and an homologous series of primary *n*-alkylamines from ethylamine to decylamine, found that the free energy and relative ease of cation exchange were direct functions of amine molecular weight. Similar results were obtained by Theng et al. (1967) for various primary, secondary, and tertiary alkylammonium cations and clay. This effect is thought to result in part from the additivity of van der Waals forces contributing to the adsorption process. The higher-molecular-weight amines might be expected to be less easily desorbed or displaced from the clay surface than the lower-molecular-weight amines by cations in the liquid. Thus, the preceding biodegradation data taken with the results of these other workers suggested that the rate of biodegradation of the bentonite–*n*-alkylamine complexes might be equal to, or limited by, the rate of desorption of the amines. Experiments were thus set to determine whether microorganisms use only that fraction of the amines which is in solution.

When a clay–organic complex is put into suspension, an equilibrium is established whereby the sorbed chemical is distributed between the complex and the surrounding solution, e.g.:

$$\text{clay–}RNH_3^+ + M^+ \rightleftharpoons \text{clay–}M^+ + RNH_3^+$$

where R is an alkyl group and M^+ is a competing cation. If microorganisms use the desorbed amine in solution, the equilibrium is shifted toward desorption. When the population of organisms is very large, the amine concentration free in solution will be effectively zero because the organisms will be utilizing the substrate as quickly as it desorbs from the clay surface, and amine desorption will be the rate-limiting step. Our approach has been to attempt to estimate the desorption rate using a purely physicochemical technique: continuous dialysis at high flow rate. Dialysis may be viewed as a simulation of microbial removal of a chemical from the suspension with a concomitant shift in equilibrium. The desorption rate can then be compared with the rate of biodegradation of the clay–amine complex with high bacterial cell densities under similar conditions. Furthermore, the biodegradation rate presumably should be independent of cell density at high bacterial densities if the amine desorption step is rate limiting. For these measurements, bentonite–*n*-[1-^{14}C]-decylamine was prepared to provide the sensitivity (ng/mL) and precision required.

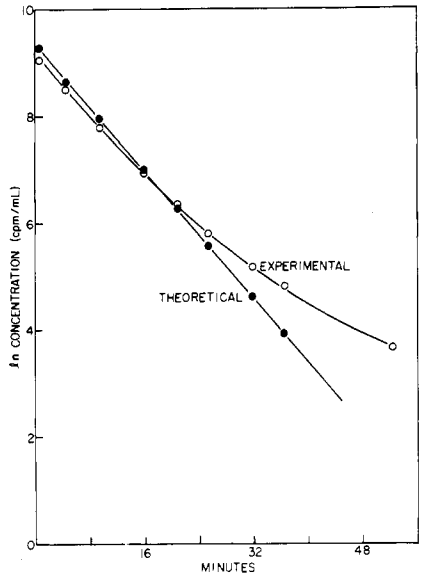

Figure 2. Exponential dilution of a solution initially containing 1 μg of *n*-[1-^{14}C]decylamine/mL of inorganic salts solution.

The ultrafiltration cell used to determine the desorption rate provides for continuous dialysis (or diafiltration) at constant flow rate and a constant sample volume. This type of cell has been used to obtain adsorption/desorption isotherms, at low flow rates, for several pesticides with soil, clay, and a humic acid preparation (Grice and Hayes, 1972; Grice et al., 1973). Selection of a suitable membrane presented a problem, however. Several membranes were tested by filtering a solution containing 0.24 μg of *n*-[1-^{14}C]decylamine hydrochloride/mL of salts solution, and it was found that a Millipore PTGC membrane and Amicon PM-10 and YM-10 membranes allowed passage of less than half of the labeled compound. Recovery of the radiolabel using the polycarbonate Nuclepore N010 (0.1 μm) membrane was considerably better: 0.19 μg/mL or nearly 80%. Moreover, a linear cpm/mL vs. concentration calibration curve was obtained for the 10 ng/mL to 10 μg/mL range of interest when various solutions of [^{14}C]decylamine were filtered through the Nuclepore membrane. Recovery of the labeled decylamine at concentrations of 0.24 μg/mL fell to about 50% using a Nuclepore (0.03 μm) membrane, presumably because the density of pores is twice that of the N010. Thus, the Nuclepore N010 membrane was selected as the best compromise for recovery of decylamine in the filtrate and for high flow rate.

As a further test of this membrane, an exponential dilution of a solution of 1.0 μg of *n*-[1-^{14}C]decylamine/mL with the inorganic salts solution was performed using the Amicon cell in the dialysis mode. Portions of the eluate were taken for liquid scintillation counting at selected times, and the resulting curve of actual concentration (cpm/mL) vs. time was compared with the theoretical curve for the concentration within the Amicon cell. The curves (Figure 2) did not correspond well, especially at lower concentrations in the Amicon cell, possibly because the initially adsorbed *n*-[1-^{14}C]decylamine was washed off the membrane to give higher cpm/mL values than predicted. This system was not abandoned, however, because it was not yet known what range of concentrations would be encountered in the actual desorption rate experiments.

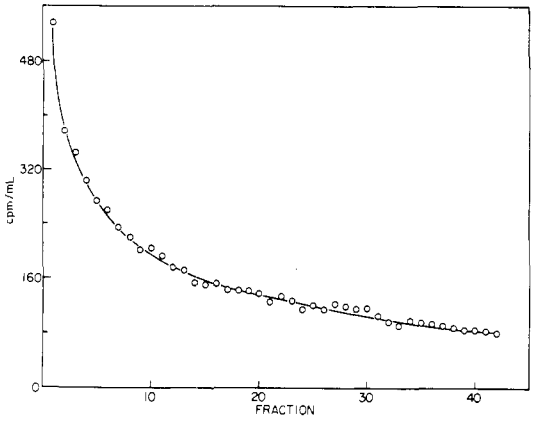

Figure 3. Desorption of *n*-[1-^{14}C]decylamine from bentonite–*n*-[1-^{14}C]decylamine suspended in an inorganic salts solution at a flow rate of 4.3 mL/min.

The amount of the suspended labeled complex (12 μg of *n*-[1-^{14}C]decylamine/mL and 0.014% solids, or less in some cases) used in the ultrafiltration cell was diluted from that used in the initial biodegradation experiments (about 100 μg/mL). The dilution was found necessary to prevent or minimize gel-polarization control of flow rate and inadequate suspension of the complex and to achieve high flow rates. The data obtained for each eluate fraction in an experiment performed at a flow rate of 4.3 mL/min are plotted in Figure 3. The total amount of *n*-[1-^{14}C]decylamine recovered during the roughly 3-h period represents only about 16% of the total bound in the complex. Nevertheless, it is clear that the number of cpm/mL per fraction desorbed from the complex had reached an approximately constant level. The constant desorption may reflect the chemical released from the inner lattice surfaces, whereas the initial, steep portion of the curve may reflect desorption of more loosely bound decylamine on external surfaces. Curves of the same shape were always obtained, regardless of the flow rate through the system, in the diafiltrations performed.

A maximum sustainable rate of amine removal was calculated for each flow rate from the approximately constant level reached at the end of each diafiltration, assuming that subsequent values would not be significantly higher but would continue to drop slowly. The calculation of removal rate is made on the basis of the flow rate, e.g., 4.3 mL/min, and the corresponding number of times the suspension volume was exchanged per hour (56 times). The limiting concentration in the final eluate fractions of Figure 3, corresponding to about 90 cpm/mL, was 14 ng/mL. The 14 ng/mL derives from the linear calibration curve obtained with the N010 membrane. Then, since the suspension volume was turned over 56 times/h, 56 × 14 ng/mL yields 784 ng of *n*-[1-^{14}C]decylamine/mL per hour desorbing from the complex. Since a relatively narrow concentration range (about 3–50 ng/mL) of *n*-[1-^{14}C]decylamine was encountered in the diafiltration experiments relative to the exponential dilution test (0–1 μg/mL) it is assumed that the values obtained closely reflect the actual cell concentration, particularly in the final stages where the concentration was fairly constant and filtration of a single or uniform solution was approximated.

To determine unambiguously the rate of desorption from bentonite–*n*-[1-^{14}C]decylamine, it is necessary to find the maximum rate of removal of the amine from the clay. If the flow rate in the ultrafiltration cell could be increased to very high levels, this maximum rate presumably would be reached and would thereafter be unaffected by increased flow rate. These diafiltration experiments were designed to find this maximum rate, or desorption rate, within the range of flow rates attainable with the ultrafiltration cell and membrane. A plot of rates of amine removal vs. cell flow rates obtained with this system is given in Figure 4. Data plotted for flow rates of 1.1, 2.3, and 4.1 mL/min were obtained with the bentonite–*n*-[1-^{14}C]decylamine suspension corresponding to a concentration of 12 μg/mL of the labeled amine and 0.014% solids. These data show that the amine removal rate was still increasing with increased flow rate. To obtain data for flow rates of 6.2 and 9.0 mL/min, the suspension was diluted by factors of 4.0 and 6.4, respectively. When these data are plotted (points A and B in Figure 4), the amine removal rate appears to drop. However, it is not clear how these two points should be compared with those obtained with the original suspension.

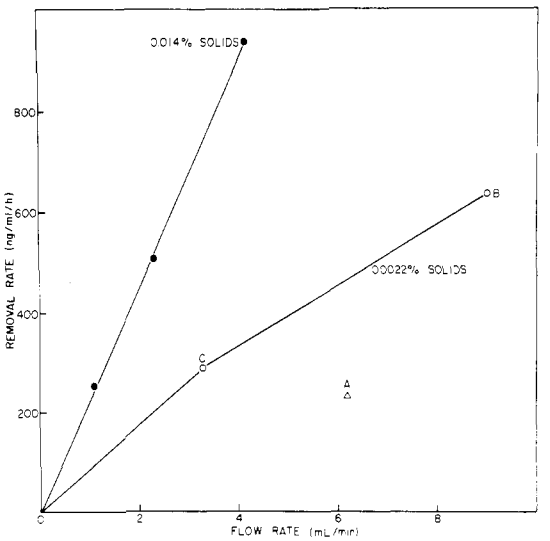

Figure 4. Rate of removal of *n*-[1-^{14}C]decylamine from bentonite–*n*-[1-^{14}C]decylamine as a function of flow rate.

To assess the validity of extrapolating from points A and B by multiplying the plotted removal rates by the dilution factors 4.0 and 6.4, the suspension diluted by 6.4 (0.0022% solids) was used at a lower flow rate, 3.3 mL/min. The actual removal rate obtained in this test is given as point C in Figure 4, about 290 ng mL^{-1} h^{-1}. If this value is multiplied by 6.4, the extrapolated rate is about 1800 ng mL^{-1} h^{-1}, substantially higher than 940 ng mL^{-1} h^{-1}, the rate obtained for 4.1 mL/min with the original suspension (0.014% solids). The plotted removal rate has increased from 3.3 to 9.0 mL/min at 0.0022% solids, but the increase was not as sharp as the observed increase from 1.1 to 4.1 mL/min at 0.014% solids. Thus, it appears likely that the value for the desorption rate was already being approached, but it is not possible to determine the actual desorption rate from the data presented.

The data obtained for the production of $^{14}CO_2$ from bentonite–*n*-[1-^{14}C]decylamine with three inocula are plotted in Figure 5. The curve labeled "free (2 × 10^8)" represents data for *n*-[1-^{14}C]decylamine in clay-free salts solution with an initial cell density of 2 × 10^8 cells/mL. It is evident that the production of $^{14}CO_2$ from the labeled complex is dependent on the inoculum size. Furthermore, the clay-bound *n*-[1-^{14}C]decylamine was converted to $^{14}CO_2$ much more slowly than the labeled amine in the clay-free solution when the initial cell density was 2 × 10^8 cells/mL.

Rates of biodegradation were calculated from the linear portions of the $^{14}CO_2$ evolution curves in Figure 5. The rate calculations take into account the percentage of the total amount (in cpm) of *n*-[1-^{14}C]decylamine that the

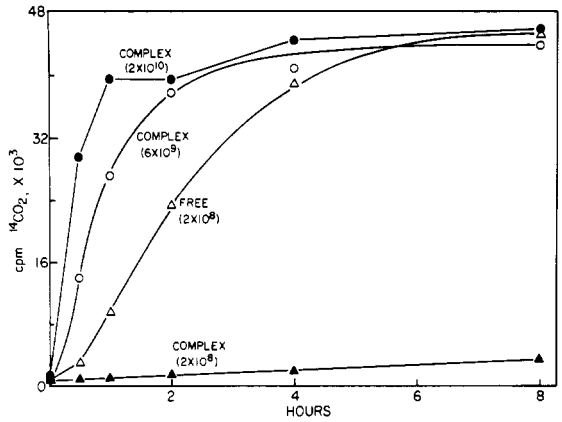

Figure 5. Evolution of $^{14}CO_2$ in the bacterial decomposition of bentonite–*n*-[1-^{14}C]decylamine complex and free *n*-[1-^{14}C]decylamine in inorganic salts solution with various initial bacterial cell densities (cells/mL).

microorganisms converted to cpm of $^{14}CO_2$, which was found to be 48% in 24 h in the clay-free solution. The calculations were based on the conversion for the clay-free solution because the total cpm in the bentonite–*n*-[1-^{14}C]decylamine solution could not be measured directly because of possible strong trapping of emitted beta particles within the clay lattice, where much of the amine was presumably located. For the clay suspensions, the calculated rates are: 10.5 μg mL^{-1} h^{-1} for 2×10^{10} cells/mL for the first 30-min period, 4.9 μg mL^{-1} h^{-1} for 6×10^9 cells/mL for the first 30 min, and 0.075 μg mL^{-1} h^{-1} for 2×10^8 cells/mL for the first 8 h. The clay-free solution with an initial bacterial density of 2×10^8 cells/mL had a mineralization rate of 2.4 μg mL^{-1} h^{-1} for the 30–120-min period.

Based on these calculations, it appears that the biodegradation rate at the two highest cell densities exceeds the highest rate of amine removal found here by factors of more than 5 and 10. These experiments were performed under the same conditions of temperature, amount of bentonite–decylamine/mL of suspension, and percent solids so that comparisons should be valid. The chief difference between the experiments involving bacteria and no bacteria was the degree of agitation of the suspension, which was much higher in the case of desorption with no inoculum. If the degree of agitation or shaking was increased for the suspensions containing the inocula, it is likely the rates of CO_2 production would have been even higher and the discrepancy between the rates of degradation and removal even larger.

These observations suggest that the desorption rate does not limit the rate of biodegradation of the bound *n*-decylamine, and the mineralization rate of this sorbed compound is not independent of inoculum size at high cell densities. The microorganisms thus may facilitate removal of the decylamine from the clay surface; e.g., by production of extracellular enzymes or by bringing about a pH change at the clay surface. Nevertheless, CO_2 production from the sorbed decylamine is markedly lower than from the free amine. The reason for this reduction in rate is not clear, but it is not the result of a toxicity of the clay because the rate of $^{14}CO_2$ production from D-[^{14}C(U)]glucose by two pure bacterial cultures isolated from the mixed culture growing on *n*-decylamine was the same in the presence and absence of bentonite (0.014% solids). D-[^{14}C(U)]Glucose did not adsorb to the clay. The bound decylamine may be degraded more slowly than the free amine because some portion or all of that which is bound on the inner clay surfaces is poorly accessible to the microorganisms.

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Received for review July 31, 1978. Accepted October 10, 1978. This research was supported by National Science Foundation Grant ENV75-19797. Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not reflect views of the National Science Foundation.

Processing of Fresh Tobacco Leaves for Protein Fractions

Benny E. Knuckles,* George O. Kohler, and Donald deFremery

A scaled-up process was developed for the preparation of crystalline fraction I protein and fraction II protein from tobacco. Laboratory tests were used to evaluate possible conditions for pilot plant processing. The plant tissue was treated with a reducing agent to inhibit undesirable oxidative reactions. Sephadex G-50 was used for large-scale gel filtration. The yield of crystalline fraction I protein was greater from leaf tissue than from stem tissue (3 g/kg of fresh leaf and 0.02 g/kg of fresh stem). Forty-five kilogram batches of fresh leaf tissue could be processed to yield 95 g of crystalline fraction I protein, which had a nitrogen content of 17.03%. The dried protein fractions were analyzed for amino acid content and soluble nitrogen.

In anticipation of increased needs for edible protein, research is being conducted on more efficient utilization of the protein that is produced by green plants. Much plant protein is converted to animal protein with considerable loss before it is consumed by humans as meat, milk, and eggs. Greater efficiency can be achieved by extracting protein concentrates from green leafy tissue and using the fibrous residue, which still contains some protein,

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