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Degradation of Substituted Indoles by an Indole-Degrading Methanogenic Consortium

JI-DONG GU AND DUANE F. BERRY*

Department of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

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Degradation of indole by an indole-degrading methanogenic consortium enriched from sewage sludge proceeded through a two-step hydroxylation pathway yielding oxindole and isatin. The ability of this consortium to hydroxylate and subsequently degrade substituted indoles was investigated. Of the substituted indoles tested, the consortium was able to transform or degrade 3-methylindole and 3-indolyl acetate. Oxindole, 3-methyloxindole, and indoxyl were identified as metabolites of indole, 3-methylindole, and 3-indolyl acetate degradation, respectively. Isatin (indole-2,3-dione) was produced as an intermediate when the consortium was amended with oxindole, providing evidence that degradation of indole proceeded through successive hydroxylation of the 2- and 3-positions prior to ring cleavage between the C-2 and C-3 atoms on the pyrrole ring of indole. The presence of a methyl group (—CH_3) at either the 1- or 2-position of indole inhibited the initial hydroxylation reaction. The substituted indole, 3-methylindole, was hydroxylated in the 2-position but not in the 3-position and could not be further metabolized through the oxindole-isatin pathway. Indoxyl (indole-3-one), the deacetylated product of 3-indolyl acetate, was not hydroxylated in the 2-position and thus was not further metabolized by the consortium. When an H atom or electron-donating group (i.e., —CH_3) was present at the 3-position, hydroxylation proceeded at the 2-position, but the presence of electron-withdrawing substituent groups (i.e., —OH or —COOH) at the 3-position inhibited hydroxylation.

Aromatic N-heterocyclic compounds, including substituted indoles, are often found in aqueous waste effluents associated with oil shale and coal mining operations (5, 14). Production of synthetic fuels from aboveground oil shale retorting processes has resulted in contamination of surface waters and groundwaters with these chemicals (23). Members of the N-heterocyclic aromatic chemical class can be toxic (26). For example, 3-methylindole has been shown to induce pneumotoxicosis in ruminants (9–12). Additionally, indolic compounds can be a nuisance because of their strong odor (4, 7). Chemical disinfection of indole-containing wastewaters with dilute aqueous solutions of chlorine, chlorine dioxide, and chloramine can result in the formation of chlorinated aromatic products (20).

Information regarding the capabilities of microbes to degrade N-heterocyclic aromatic compounds should prove useful in determining the fate of these chemicals in the environment. Since anaerobic conditions prevail in many sediment and subsurface environments, it is important to examine the anaerobic biodegradation potential of these chemicals.

The metabolism of heterocyclic aromatic compounds under methanogenic conditions has not been extensively investigated (2). Of the indolic compounds investigated, only indole has been examined to any significant extent. Recent reports by Berry et al. (3) and Madsen et al. (22) indicated that addition of indole to methanogenic sewage sludge and sediment serum bottle microcosms resulted in production of the hydroxylated metabolite, oxindole, the initial transformation intermediate. Not surprisingly, the ability of sediment and sewage sludge microcosms to degrade indole was dependent upon several factors, including incubation temperature and the amount of sediment or sludge inoculum used (22).

Most studies concerning fermentation of indolic compounds have concentrated on either transformation of substituent groups (27) or ring metabolism (1, 3, 21). None have investigated the effect of substituent group substitution on indole hydroxylation reactions. This investigation evaluated the effects of substituent groups (i.e., —CH_3, —COOH, and —OH) on the initial ring hydroxylation and subsequent mineralization reactions. An indole-degrading methanogenic consortium enriched from sewage sludge was used in this substrate specificity study.

MATERIALS AND METHODS

Chemicals. Indolic compounds used in this investigation, including indole, 1-methylindole, 2-methylindole, 3-methylindole (skatole), 3-indolyl acetate, indole-3-carboxylic acid, oxindole, isatin, and indigo (purity, 97 to 99%), were purchased from Aldrich Chemical Co., Inc. (Milwaukee, Wis.), and used without further purification. The chemical 3-methyloxindole, a metabolite of 3-methylindole, was isolated from CH_2Cl_2 extracts of culture filtrate by thin-layer chromatography. The enrichment culture was amended with 3-methylindole on an intermittent basis for over 90 days. Approximately 1 mg of purified 3-methyloxindole was obtained from 0.5 liter of culture filtrate. The identity of this metabolite was confirmed on the basis of mass spectrometry, producing a mass spectrum with a strong molecular ion peak at *m/z* 147 (see Fig. 4a), UV spectroscopy (λ_{max}, 249 nm [17]), and proton nuclear magnetic resonance (^{1}H-NMR) spectra: ($CDCl_3$), δ 1.52 (d, CH_3, J = 7.7 Hz), 3.48 (q, H-3, J = 7.6 Hz), 6.92 (d, H-7, J = 7.9 Hz), 7.05 (dd, H-6, J = 7.0 Hz, J = 7.9 Hz), 7.23 (dd, H-5, H-4, J = 6.4 Hz, J = 7.9 Hz). (Refer to the 3-methyloxindole structure in Fig. 4a for H assignments.) The authenticated 3-methyloxindole standard

* Corresponding author.

was obtained in this manner because it was not commercially available.

Microorganisms and culture conditions. A methanogenic indole-degrading enrichment culture containing four to six recognizable morphological types was enriched from anaerobic municipal sewage sludge (Roanoke, Va.). Enrichment culture continuous-transfer techniques, using indole as the sole carbon and energy source, were used to maintain the indole-degrading consortium. Sludge (1 liter) was placed in a tightly stoppered 2-liter flask. Indole (0.43 mmol dissolved in 0.1 ml of methanol) was added to the culture vessel on an intermittent basis as required to maintain the initial indole concentration (0.43 mM). The substrate (indole) concentration was determined by high-performance liquid chromatography (HPLC) analysis of culture filtrate. The enrichment culture was incubated in the dark at room temperature for 190 days. Following the enrichment process, 25 ml of the sewage sludge culture was transferred to 160-ml serum bottles containing 75 ml of deoxygenated mineral salts medium. The mineral salts medium (containing 1 ml of 0.1% resazurin solution) was prepared as described by Boyd et al. (6). The medium, after being autoclaved for 15 min, was maintained under N_2 that was previously passed through hot (300°C) copper filings to remove traces of O_2. Indole was added after the medium had cooled to 45°C. During transfer of the sewage sludge inoculum, serum bottles were maintained under a positive pressure of O_2-free N_2. Serum bottles were closed with thick butyl rubber stoppers and aluminum crimp seals. The indole-degrading consortium was transferred once every 10 days (approximately) on the basis of depletion of indole and oxindole by transferring 20 ml of the indole-depleted culture to serum bottles containing 80 ml of fresh mineral salts medium (plus indole). Cultures were transferred a total of 44 times prior to use in biodegradation studies.

Experiments were initiated by transferring 20 ml of indole-oxindole-depleted culture medium to 80 ml of fresh mineral salts medium (in 160-ml serum bottles) containing between 21 and 43 μmol of indolic compound. Strictly anaerobic conditions were maintained throughout the transfer process. Batch fermentation cultures were incubated under static conditions in the dark at 24°C. All fermentation experiments were conducted in triplicate. Samples (1 ml) of culture medium were periodically withdrawn by syringe and stored frozen in glass vials until analyzed. Additional serum bottle microcosms (three) were initiated and sacrificed in the event that a metabolite was detected during the fermentation trial. Controls consisted of unamended (no indole addition) and autoclaved cultures. The difference in methane production between amended and unamended controls was used to determine mineralization potential. Microcosms used as sterile controls were autoclaved for 20 min on each of three successive days. For the sterile control microcosms, indolic compounds (dissolved in 500 μl of methanol) were added to the medium following passage through a 0.2-μm-pore-size syringe filter.

Analysis. In preparation for HPLC analysis, culture samples were thawed, mixed with methanol (1:1), centrifuged (13,000 × *g*), and filtered through Gelman (Ann Arbor, Mich.) 0.2-μm Acrodisc membrane filters. Methanol was used in the sample preparation to ensure that neither indoles nor metabolites adsorbed to membranes.

Samples were chromatographed on an HPLC system (LDC Analytical, Riviera Beach, Fla.) consisting of a CM 3400 pump and a 3100 variable-wavelength spectrophotometer. Separation of parent indolic compounds and metabolites was accomplished by using a 25-cm Supelcosil 5-μm-particle-size LC-18-DB column.

Methanol-water (60:40, vol/vol) delivered at a flow rate of 1 ml min^{-1} was used as the mobile phase in the HPLC analysis of indole, 1-methylindole, 2-methylindole, oxindole, and isatin. The mobile phase for separation of 3-methylindole, 3-methyloxindole, 3-indolyl acetate, and indole-3-carboxylic acid consisted of a 25:10:65 mixture of methanol–water–acetonitrile-water-acetic acid (60:39.5:0.5, vol/vol/vol) at a flow rate of 1 ml min^{-1}. Indole, 3-methylindole, 2-methylindole, 1-methylindole, indole-3-carboxylic acid, 3-indolyl acetate, oxindole, 3-methyloxindole, and isatin were quantified by the external standards method at wavelengths of 271, 290, 270, 260, 260, 273, 247, 249, and 243 nm, respectively. The calibration curves were linear for these compounds in the range of 0.05 to 0.5 mM.

The amount of methane produced in serum bottle microcosms was determined by injecting 50 μl of the headspace gas into a gas chromatograph (model 5890; Hewlett Packard Co.) equipped with a thermal conductivity detector and fitted with a Porapak N column (1.8 m by 2.1 mm internal diameter, 80/100 mesh). The column temperature was maintained at 50°C, and the detector temperature was maintained at 150°C. The flow rate of the carrier gas, helium, was 20 ml min^{-1}.

Metabolite isolation and identification. The contents of replicate serum bottles were filtered (no. 1 filter; Whatman, Inc., Clifton, N.J.) and extracted three times with 25 ml of CH_2Cl_2. The extracts were combined and evaporated to dryness in a flash evaporator. Residue was dissolved in 5 ml of CH_2Cl_2, dried by passage through Na_2SO_4, and transferred to a conical glass tube, and the volume was reduced to 0.2 ml under N_2. Thin-layer chromatography using 0.25-mm-thick precoated silica gel plates (no. 06-600C, Fisherbrand; Fisher Scientific, Springfield, N.J.) was used to separate components. The mobile phase used to separate the indole metabolite (R_f = 0.46) consisted of hexane-CH_2Cl_2-ethyl acetate (6:1:3, vol/vol/vol). To separate the 3-methylindole metabolite (R_f = 0.62), CH_2Cl_2-methanol (100:1, vol/vol) was used, and to separate the oxindole metabolite (R_f = 0.69) hexane-CH_2Cl_2-ethyl acetate (6:1:6, vol/vol/vol) was used. The metabolites were extracted from silica gel with methanol. A portion of the methanol extract containing either the indole, 3-methylindole, or oxindole metabolites was placed in a small vial and evaporated to dryness under N_2 in preparation for mass spectrometry. The remaining portion of methanol extract was used to determine the metabolite UV spectrum.

Indigo, the dimerization product of the 3-indolyl acetate metabolite, indoxyl, was separated by thin-layer chromatography using a $CHCl_3$-glacial acetic acid (19:1, vol/vol) mobile phase. Methylene chloride was used to extract indigo from the silica gel. A portion of the indigo was evaporated to dryness under N_2 in preparation for mass spectrometry, while the remaining CH_2Cl_2 extract was used to determine the UV-visible absorbance spectrum.

A Beckman DU-7 scanning spectrophotometer (Beckman Instrument, Inc., Irvine, Calif.) was used to determine UV-visible spectra. Mass spectral data used to confirm the identification of indole, 3-methylindole, and oxindole metabolites and of indigo were obtained by using a 7070E-HF high-resolution mass spectrometer (VG Analytical, Manchester, United Kingdom) with a direct insertion probe at an electron energy of 70 eV. A Bruker 270-MHz spectrometer was used to determine NMR spectra. Deuterated chloroform was used as a solvent.

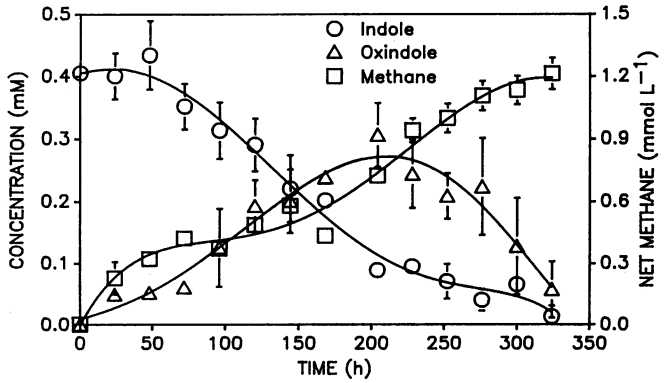

FIG. 1. Metabolism of indole with subsequent production of oxindole and methane. Concentration data (mean ± standard deviation) showing the disappearance of indole, the appearance and disappearance of oxindole, and the appearance of methane were fit to a fifth-order polynomial regression model ($r^2 \geq 0.95$).

RESULTS

Indole metabolism. Following a 50-h lag period, the indole-degrading methanogenic consortium degraded indole within 325 h (Fig. 1). No appreciable losses of indole were observed in sterile controls. Metabolite appearance correlated positively with indole disappearance. On the basis of results from earlier investigations (3, 21), oxindole (1,3-dihydro-2H-indol-2-one) was suspected to be the metabolite. The metabolite was identified as oxindole by comparison with an authentic oxindole standard by using UV absorbance (λ_{max}, 247 nm), HPLC retention time (3.43 min), and mass spectral analysis (*m/z*, 133). The oxindole concentration in the microcosms reached a maximum level when approximately 75% of the substrate, indole, had been metabolized. After 325 h of incubation, net methane production in serum bottle microcosms came close (78%) to that predicted by the stoichiometric relationship $C_8H_7N + 7H_2O \rightarrow 4.5CH_4 + 3.5CO_2 + NH_3$, providing evidence that indole was mineralized.

Madsen and Bollag (21) recently proposed that indole degradation, under denitrifying conditions, proceeds through a series of transformation steps beginning with the hydroxylation of indole, forming oxindole, followed by a second hydroxylation step leading to the formation of isatin (indole-2,3-dione). The results of that study prompted us to evaluate indole degradation under strictly anaerobic conditions by using our methanogenic indole-degrading consortium. Small amounts of isatin in culture filtrate were sometimes detected, suggesting that the indole-degrading methanogenic consortium was using the oxindole-isatin pathway. When oxindole (authentic) was supplied to the indole-degrading methanogenic consortium as the sole source of carbon and energy, this compound was readily consumed and isatin was produced. Mass spectral analysis (*m/z*, 147; Fig. 2), UV absorbance (λ_{max}, 243 nm), and HPLC retention time (3.23 min) of the metabolite were identical to those of an authentic isatin standard. Disappearance of oxindole or appearance of isatin was not observed in autoclaved sterile controls.

Fate of methylindoles. Methyl group substitution in the 1-, 2-, or 3-position had a marked influence on degradability. While the consortium was unable to degrade 1-methylindole or 2-methylindole, a substantial amount of 3-methylindole was converted to a metabolite after a 100-h lag period (Fig. 3). The metabolite, first observed as an unidentified peak in

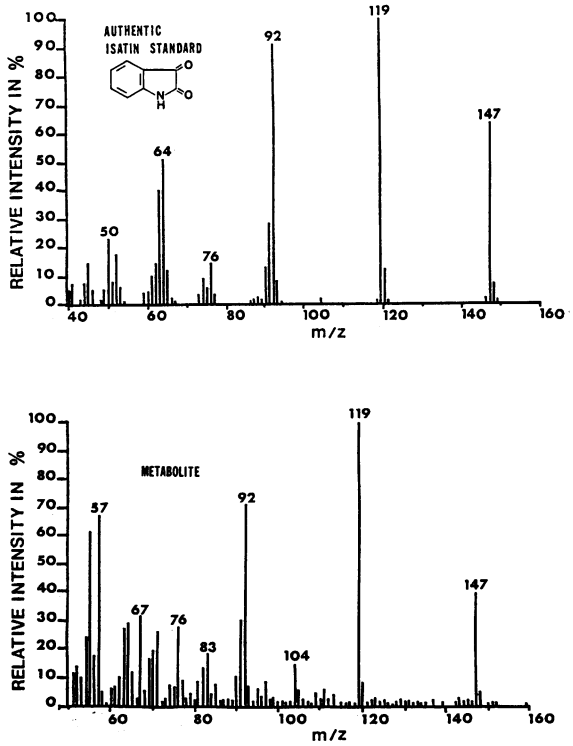

FIG. 2. Mass spectra of authentic isatin and a metabolite isolated from batch cultures containing oxindole and the indole-degrading consortium.

HPLC chromatograms of culture filtrates, was identified as 3-methyloxindole (1,3-dihydro-3-methyl-2H-indol-2-one) on the basis of mass spectral analysis (Fig. 4b), HPLC retention time (4.10 min), and UV absorbance (λ_{max}, 249 nm [17]). We did not observe the disappearance of 3-methylindole or the appearance of 3-methyloxindole in the sterile controls. The indole-degrading methanogenic consortium was unable to mineralize 3-methylindole on the basis of the fact that net methane production was negligible even after 600 h of

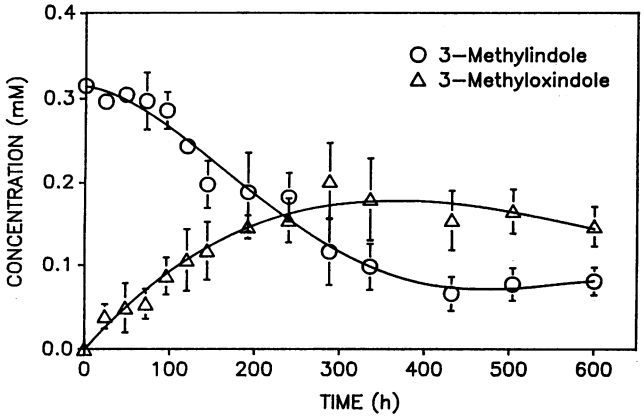

FIG. 3. Transformation of 3-methylindole with subsequent production of 3-methyloxindole. Concentration data (mean ± standard deviation) showing disappearance of 3-methylindole and appearance of 3-methyloxindole were fit to fourth-order and third-order polynomial regression models, respectively ($r^2 \geq 0.96$).

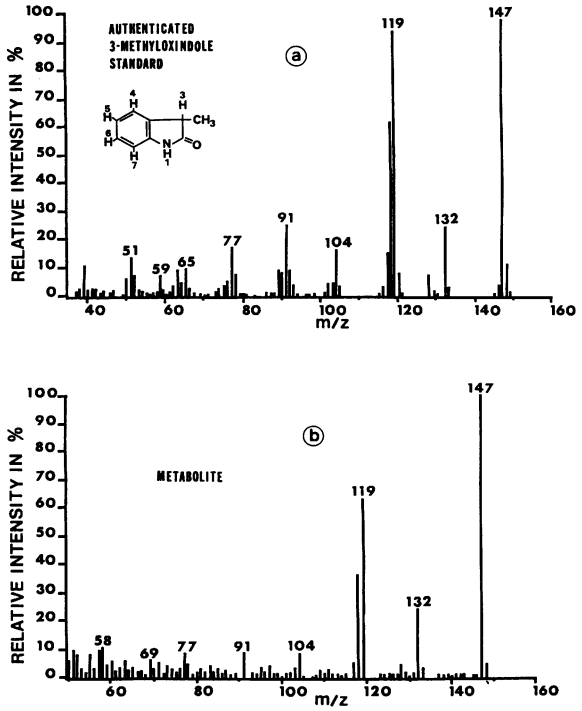

FIG. 4. Mass spectra of an authenticated 3-methyloxindole standard (a) and a metabolite isolated from batch cultures containing 3-methylindole and the indole-degrading consortium (b).

incubation. Additionally, efforts to sustain the indole-degrading consortium by using 3-methylindole failed, indicating that this compound could not serve as a carbon and energy source. Production of 3-methyloxindole in 3-methylindole fermentation tests probably resulted from nutrient carryover with the transfer of indole-grown cell inoculum. Also, indole hydroxylase(s) may have been active transiently in transferred cells.

Fate of 3-indolyl acetate and indole-3-carboxylic acid. The effect of substituent groups at the 3-position of indole was examined further by supplying indole-degrading cultures with either 3-indolyl acetate or indole-3-carboxylic acid. We had intended to examine the ability of the indole-degrading consortium to degrade indoxyl (1,2-dihydro-3H-indol-3-one); however, a commercial source of this chemical could not be located. It was anticipated that 3-indolyl acetate would be an alternative source. We envisioned degradation of 3-indolyl acetate to occur via an initial facile deacetylation, yielding indoxyl. In fact, we observed that 3-indolyl acetate underwent an initial transformation quite rapidly without any apparent lag period (Fig. 5). By comparison, the lag period noted previously for indole degradation was 50 h, whereas the lag period for 3-methylindole was twice that of indole, or about 100 h (Fig. 1 and 3). Although one-third of the 3-indolyl acetate was degraded within 48 h, approximately 480 h was required for complete degradation to occur. Disappearance of 3-indolyl acetate in autoclaved sterile controls was negligible.

Examination of HPLC chromatograms of culture filtrate revealed the presence of a peak, possibly representing the intermediate metabolite, indoxyl. Efforts to isolate indoxyl were unsuccessful because of its instability in the presence of O_2. The unidentified metabolite readily accumulated in

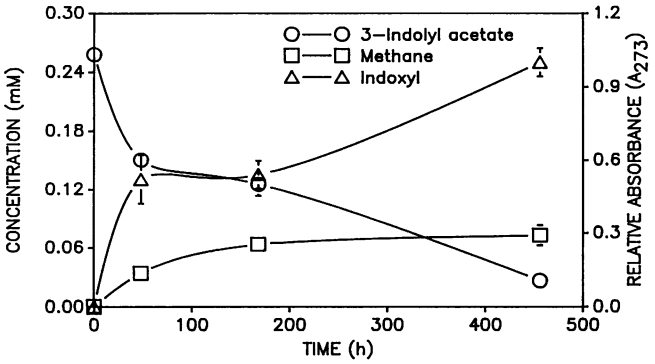

FIG. 5. Degradation of 3-indolyl acetate with subsequent production of the proposed metabolite, indoxyl, and methane (concentration mean ± standard deviation). Indoxyl concentration in culture medium is represented by a relative absorbance (A_{273} mean ± standard deviation) scale.

anaerobic culture medium and had a HPLC retention time identical to that of oxindole (3.73 min). On the basis of structural similarity, we would have anticipated similar retention times for these two compounds. While there is some question as to whether the unidentified HPLC chromatogram peak represented indoxyl, there is little doubt that this compound was present in the culture medium. After several days of incubation, we observed development of a light blue color in the cultures, indicating the formation of indigo, which spontaneously arises from dimerization of two indoxyl molecules (13, 24). The UV-visible absorbance of the blue pigment (λ_{max}, 284, 600 nm), R_f value (0.61), and mass spectral analysis (Fig. 6) compared favorably with those of an authentic indigo standard. It is probable that at least some of the indoxyl, present in the culture medium, dimerized (forming indigo) during extraction since no effort was made to exclude O_2 from this procedure.

Net differences in methane production between 3-indolyl acetate-amended and unamended cultures were very small. Theoretically, less than 10% of the acetate group cleaved from 3-indolyl acetate ended up in the form of methane (Fig. 5). Net differences were expected because the indole-degrading consortium readily produced methane in excess of that produced in unamended controls following addition of acetate to cultures (25a). These results strongly suggest that the aromatic ring of the 3-indolyl acetate was not mineralized. Indole-3-carboxylic acid was not degraded by the consortium.

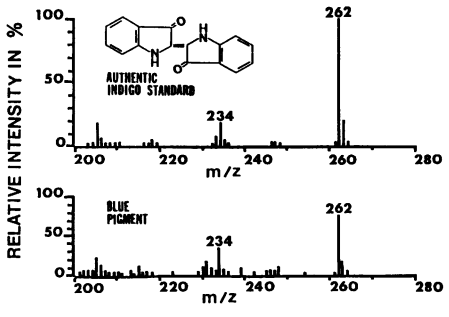

FIG. 6. Mass spectra of authentic indigo and a blue pigment isolated from batch cultures containing 3-indolyl acetate and the indole-degrading consortium.

DISCUSSION

Madsen and Bollag (21) proposed an indole degradative pathway for denitrifiers that includes two successive hydroxylation steps resulting in the formation of isatin. They further proposed that isatin is ring cleaved between the C-2 and C-3 atoms and not between the N-heteroatom and the adjacent carbon atom, as would be the case for ring cleavage of other N-heterocyclic aromatic compounds, including nicotinic acid (25), uracil (8), and purine (15). In another study, Claus and Kutzner (13) proposed a pathway for the catabolism of indole by *Alcaligenes* strain In3 under aerobic conditions that was similar to that proposed by Madsen and Bollag (21). Claus and Kutzner's pathway differed in that the initial hydroxylation reaction occurred first at the 3-position (forming indoxyl) and was followed by a second hydroxylation at the 2-position (forming isatin). Because indoxyl and isatin were never actually isolated from the actively indole-degrading cultures, the investigators were unable to substantiate their proposed indoxyl-isatin pathway. The presence of indigo in the culture medium was offered as indirect evidence supporting indoxyl formation. No effort was made by Claus and Kutzner (13) to determine the source of oxygen (i.e., molecular oxygen or water) used in the enzyme-mediated oxidation of indole or indoxyl.

In a recent investigation, Kamath and Vaidyanathan (19) reported that degradation of indole by *Aspergillus niger* is initiated by hydroxylation at the 3-position (forming indoxyl), followed by a ring cleavage reaction forming N-formyl anthranilate. The production of indigo in culture medium was used to support these investigators' contention that indole was first transformed to indoxyl prior to the ring cleavage reaction. Indigo formation was attributed to the instability of indoxyl in the presence of O_2. O_2 is apparently not absolutely required for the formation of indigo because this compound was produced during the degradation of 3-indolyl acetate by our methanogenic indole-degrading consortium. We did observe, however, an intensification of blue color formation in the culture medium when the anaerobic cultures were exposed to O_2. Attempts to demonstrate indole oxygenase activities were unsuccessful (19). Though indole degradation pathways under aerobic and anaerobic conditions have similarities, including an initial hydroxylation step and the ring cleavage between the C-2 and C-3 atoms, there is an interesting difference between the pathways. Under aerobic conditions, the initial hydroxylation occurs either at the 3-position (13, 19) or simultaneously at the 2- and 3-positions (16). Under methanogenic conditions, the initial hydroxylation occurs at the 2-position, as shown in the present study as well as in an earlier investigation (3) and under denitrifying conditions (21).

Substitution of the hydrogen with a $—CH_3$ group at the 3-position of an indole could make it difficult to hydroxylate that position, which, according to the proposed oxindole-isatin pathway, would be requisite for continued anaerobic metabolism. Indeed, it would be interesting to isolate a 3-methylindole-degrading anaerobe to help establish the oxindole-isatin pathway as a universal pathway for fermentation of indoles.

Berry et al. (2) have suggested that protonation of indole at the 3-position (forming the 3H-indolium ion) activates the 2-position, rendering it susceptible to a nucleophilic attack (hydroxylation). Under anaerobic conditions, the source of oxygen for the hydroxylation reaction is water (a nucleophile), as is the case for hydroxylation of nicotinic acid (18). If this is the operative mechanism for the hydroxylase system(s) present in our indole-degrading consortium, we would anticipate that substituent groups attached to the pyrrole ring that can stabilize the positive charge at the 2-position of the conjugate acid will also stabilize the parent base relative to the conjugate acid. On the basis of this premise, we would predict the following reactivity or biodegradability sequence for indolic compounds: 2-methylindole (pK_a, −0.28) > 1-methylindole (pK_a, −2.3) > indole (pK_a, −3.6) > 3-methylindole (pK_a, −4.6) > indole-3-carboxylic acid (pK_a, −6.13). Electron-donating substituent groups (i.e., $—CH_3$) located at either the 1- or 2-position would help to stabilize the indolium ion through direct inductive effects, whereas substitution of electron-donating substituent groups at the 3-position would only be effective at stabilizing the indolium ion through indirect inductive effects. Electron-withdrawing substituent groups (i.e., —COOH and —OH) located at either the 1- or 3-position would destabilize the indolium ion through either direct or indirect inductive effects and thus decrease the likelihood of pyrrole ring hydroxylation.

While it is difficult to compare the rates of indole and 3-methylindole hydroxylation directly in batch culture systems, and because there was a small difference in starting concentrations (0.1 mM), we can conclude that the consortium transformed these two compounds at relatively similar rates, at least initially. The inability of our consortium to transform 1-methylindole was unexpected. On the basis of basicity, we would have predicted that 1-methylindole would have been the compound most readily hydroxylated at the 2-position. Apparently, the indole hydroxylase(s) present in the indole-degrading consortium required that the 1-position (nitrogen atom) be unsubstituted, possibly because of a requirement for H bond formation during the catalytic event.

The observation that 2-methylindole was not transformed is not too surprising because the initial requirement for ring degradation under anaerobic conditions appears to be ring hydroxylation at the 2-position (3, 21, 22). Though it is mechanistically feasible for hydroxylation to occur at the 2-position, it would seem unlikely that the resulting intermediate formed would be stable because the presence of the $—CH_3$ group at the 2-position would prevent tautomerization from the hydroxyl form to the more stable oxo form.

The inability of the indole-degrading consortium to mineralize 3-indolyl acetate is interesting in view of the fact that hydroxylation of the 3-indolyl acetate metabolite, indoxyl, would yield isatin. Apparently, the presence of oxygen, a strong electron-withdrawing substituent group, at the 3-position inhibited hydroxylase activity. The acetate group cleaved from the pyrrole moiety serves as the substrate for the methanogenic member(s) of the indole-degrading consortium.

We found that the indole-degrading methanogenic consortium was capable of transforming 3-methylindole and 3-indolyl acetate (Fig. 7). In neither case were the aromatic ring structures catabolized. Indolic compounds not transformed by the consortium included 1-methylindole, 2-methylindole, and indole-3-carboxylic acid. Whereas studies involving enriched anaerobic consortia provide important information regarding the ability of anaerobes to degrade indoles, definitive answers to questions concerning chemical structure versus enzymatic activity will be elucidated only when investigations can be conducted with cell-free enzyme preparations.

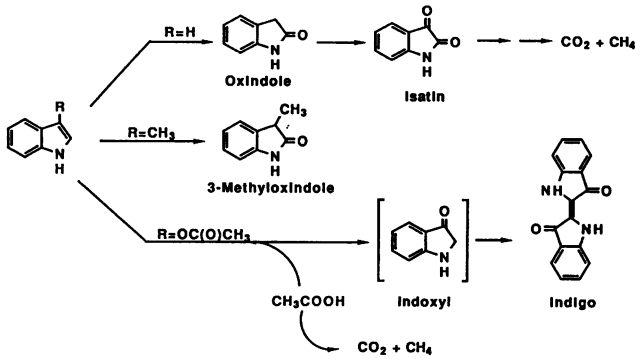

FIG. 7. Proposed pathways for degradation of indole, 3-methylindole, and 3-indolyl acetate by an indole-degrading methanogenic consortium.

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