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Anaerobic Biodegradation of Indole to Methane

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Methane gas was produced from a laboratory, granular activated carbon, anaerobic filter treating a synthetically prepared mixture of polycyclic *N*-aromatic compounds. The biodegradability of the individual polycyclic *N*-aromatic compound present in the mixture was investigated. Experimental results obtained from test bottles containing methanogenic enrichment cultures suggested that indole was degraded to methane and carbon dioxide under strict anaerobic conditions.

Aromatic compounds are contained in waste products from many industrial processes, especially industries involved in thermal processing of organic material, such as coal gasification, petroleum refining, and coke production. The discharge of these compounds causes excessive environmental damage and, consequently, they must be removed from the effluent streams. Many of these compounds have been found to be biodegradable under aerobic conditions. In recent years, a number of studies have shown that under anaerobic conditions, some of these compounds may be biodegraded to methane and carbon dioxide.

Tarvin and Buswell (10) were the first to report on the methanogenic fermentation of aromatic compounds. In their studies, they demonstrated the complete degradation of banzoic, phenylacetic, hydrocinnamic, and cinnamic acids as well as the partial decomposition of *o*-phthalic acid, salicyclic acid, phenol, and benzyl alcohol. Fina and Fiskin (3), using benzoic acid specifically labeled in the C_1 and C_7 positions, showed that the ring carbon (C_1) and the carboxyl carbon (C_7) were converted to methane and carbon dioxide, respectively. Chimielowski et al. (2) demonstrated the methanogenic biodegradability of several monohydric and polyhydric phenols, naphthols, and related aromatic compounds, whereas Healy and Young (5) reported on the complete conversion of phenol and catechol to methane and carbon dioxide. In subsequent studies, Healy and Young (6) presented evidence of the complete biodegradation to methane and carbon dioxide of 11 aromatic lignin derivatives.

Harary (4) was first in demonstrating the anaerobic degradation of a nitrogen-containing aromatic compound. He showed that the monocyclic *N*-aromatic compound nicotinic acid was fermented to acetate, propionate, ammonia, and carbon dioxide by a *Clostridium*. However, the anaerobic biodegradation of the polycyclic *N*-aromatic compound indole has not been observed previously. This report presents evidence that indole is biodegraded to methane and carbon dioxide under strict anaerobic conditions.

The defined medium for the enrichment cultures was prepared by the procedure used by Owen et al. (8) and Balch et al. (1). Four 125-ml serum bottles were purged at a flow rate of ca. 0.5 liter/min for 10 min with a mixture of 30% CO_2–70% N_2 before the introduction of the culture medium or sample. Oxygen in the purge gas mixture was removed by passing this gas through a heated (450°C) silica glass tube filled with light copper turnings (Sargent-Welch Scientific Co., Skokie, Ill.). A 100-ml volume of prereduced defined medium was transferred anaerobically to each bottle by the method of Owen et al. (8). Subsequently, activated carbon inocula ranging in weight from 2 to 4 g were added to each bottle. Finally, 17.16 mg of indole was added to each of two bottles as substrate. Two bottles remained as controls. The final liquid-phase volume in every bottle was 110 ml. The serum bottles were then sealed with butyl rubber stoppers and shaken at 35°C.

Duplicates were run for all samples, including the controls, which contained only the defined medium and the activated carbon inoculum. The polycyclic *N*-aromatic compound indole was the sole source of organic carbon that was added to the serum bottles. The defined medium was buffered at pH 7 with sodium bicarbonate in solution under a 30% CO_2–70% N_2 gas mixture. Resazurin was used as an indicator of reduced conditions. Sodium sulfide and L-cysteine hydrochloride were each added to 0.5 g/liter to provide a reducing environment.

Gas volume measurements and samplings were made by the syringe method of Nottingham and Hungate (7). Gas composition was determined with a gas partitioner (model 1200; Fisher Scientific Co., Chicago, Ill.) and certified calibration standards. The volumetric methane production data shown in Fig. 1 have been corrected for moisture content and converted to standard temperature and pressure (0°C and 1 atm [101.3 kPa]). These data also include the methane content of the liquid as determined from Henry's law and the partial pressure of the methane in the gas phase.

The concentration of the polycyclic *N*-aromatic substrate was measured on aqueous samples filtered through a membrane filter (pore size, 0.45 μm). These samples were alkalified to pH 12 and extracted with ether. The solvent phase was analyzed with a Hewlett-Packard model 5750 gas chromatograph with a glass coil column (10 ft long [ca. 3.05 m]) packed with 10% OV-101 on 80/100 Supelcoport. The oven temperature was maintained at 160°C. Both injection port and detector temperatures were set at 200°C. The granular activated carbon inoculum used in the batch test was obtained from a laboratory, granular activated carbon, anaerobic filter as described by Suidan et al. (9). This anaerobic filter had been operated continuously on a synthetically prepared feed containing indole, quinoline, and methylquinoline as well as growth nutrients for a period of 117 weeks before the carbon inoculum was taken. Gas

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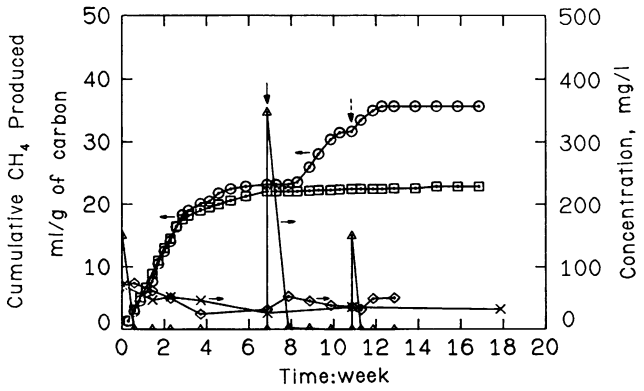

FIG. 1. Correlation between methane production and indole reduction in the enrichment cultures. Symbols: ⊙ and ⊡, average cumulative milliliters of CH_4 produced per gram of carbon from the two indole bottles and the two control bottles, respectively; △, indole concentration (milligrams per liter) in the indole bottle; ◈ and ×, methylquinoline concentration (milligrams per liter) in the indole bottle and in the control bottle, respectively; ↓, time at which indole bottles were spiked with 347 mg of indole per liter; and ⇣, time at which indole bottles were spiked with 150 mg of indole per liter.

production was not observed from the anaerobic filter until week 20. This represented the time required for acclimation plus accumulation of substrate on the carbon. The concentrations of indole, quinoline, and methylquinoline present in the feed mixture were increased from the initial concentrations of 42, 45, and 42 mg/liter, respectively, to the final concentrations of 289, 290, and 288 mg/liter, respectively. The concentrations of these compounds in the effluent remained undetectable via gas chromatography until week 104, when an effluent methylquinoline concentration of ca. 1 mg/liter was measured. Subsequently, the effluent methylquinoline concentration continued to increase, reaching a level of 130 mg/liter during week 117. Effluent quinoline and indole concentrations of ca. 1 mg/liter were detected during weeks 110 and 114, respectively. By week 117, the concentration of quinoline had increased to 4.2 mg/liter, and the concentration of indole was 2.3 mg/liter.

Because extremely low concentrations of biological solids were found in the effluent of the anaerobic filter throughout the entire study, an inoculum of activated carbon was withdrawn and transferred to serum bottles for use in the experiments on the biodegradability of the individual polycyclic *N*-aromatic compounds present in the feed mixture. Table 1 shows the size of the carbon inoculum used and the mass of indole added to each of the test bottles during this study. Figure 1 presents the methane production and the reduction of indole with time under strict anaerobic conditions. Methane gas production from the control bottles is also shown in this figure.

Methane gas was produced in the controls and in the bottles containing indole almost immediately after the initiation of the experiment. The methane produced from the control bottles leveled off after 8 weeks at an average cumulative plateau level of 22.5 ml/g of activated carbon added with the inoculum. Methane production from the indole bottles followed a similar pattern. Methane production averaged 23.2 ml/g of activated carbon at standard temperature and pressure after 7 weeks of incubation. The volume of methane produced from the indole and control bottles far exceeded the maximum potential of the indole added to the indole bottles during the first phase (weeks 0 through 7) of this study (Table 1). Since the concentration of indole in the anaerobic filter effluent was 2.3 mg/liter when the carbon inoculum was withdrawn, a significant mass of indole was expected to be adsorbed on the carbon surface. Therefore, the appreciable volume of methane production before week 7 may be attributed to the degradation of indole and other organic nutrients that were adsorbed on the activated carbon inoculum while it was serving as a contact medium in the anaerobic filter.

A lag in methane production of ca. 1 week occurred after the addition of 347 mg of indole per liter into each of the indole bottles during week 7. The lag may be due to the low activity of the indole-utilizing bacteria after a several-week period of deprivation of the indole substrate (as evidenced by the cessation of methane production in Fig. 1) or it may be due to the initial adsorption of indole by carbon after that carbon had been regenerated by biological utilization of indole or other organic nutrients during the first phase of this study. However, a third spike of indole (150 mg/liter) on week 11 resulted in an immediate increase in methane gas production. Methane production continued for a long period despite the quick disappearance of indole from the liquid phase after each spike. The prolonged methane production may be due to the desorption of previously adsorbed indole from the activated carbon surface or the utilization of indole fermentation products.

Methylquinoline was also detected in both the control bottles and the test bottles. The appearance of methylquinoline in the liquid may be attributed to desorption from activated carbon inoculum after it was transferred from the anaerobic filter to serum bottles. Parallel tests on the anaerobic utilization of methylquinoline revealed that this compound resisted biological breakdown.

The anaerobic degradation of indole to methane, carbon dioxide, and ammonia can be stoichiometrically described as follows: $C_8H_7N + 8H_2O \rightarrow 3.5CO_2 + 4.5CH_4 + NH_4^+ + OH^-$. Based on the amount of indole added, the above stoichiometric relationship, and subtracting the methane produced in the control, the theoretical methane production

TABLE 1. Weight of activated carbon inoculum used and indole added to serum bottles

Serum bottle[a]	Activated carbon inoculum added (g)	Indole added (mg)[b]		
		Wk 0	Wk 7	Wk 11
Test bottle no. 1	4.03	17.16 (15.2)	34.7 (30.2)	14.0 (12.1)
Test bottle no. 2	3.44	17.16	34.7	14.0
Control bottle no. 1	2.18	0	0	0
Control bottle no. 2	2.57	0	0	0

[a] Total liquid volume (milliliters) for each bottle: week 0, 110; week 7, 100; and week 11, 90.
[b] Values in parentheses represent the maximum methane potential (milliliters) of the added indole.

TABLE 2. Summary of methane production from serum bottles containing enrichment cultures

Period (wk)	Indole added to indole bottle (mmol)	Actual methane production (mmol)		Excess methane production in indole bottle over control bottle (mmol)	Theoretical methane production (mmol)
		Indole bottle	Control bottle		
0–7	0.15	3.87	3.70	0.17	0.68
7–11	0.30	1.42	0.07	1.35	1.35
11–18	0.12	0.67	0.02	0.65	0.54

at standard temperature and pressure can be calculated and compared with the measured values. The results show that by the end of this test, the measured methane production was 84.4% of the theoretical or potential value (Table 2). The less-than-theoretical methane production before week 7 suggests that part of the added indole was adsorbed by activated carbon. Biological utilization of adsorbed indole later occurred as evidenced by the higher methane production after the third spike (weeks 11 through 18). This clearly demonstrates that the polycyclic *N*-aromatic compound indole is fermented to CH_4 and CO_2.

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