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Anaerobic degradation of phenol in sewage sludge

Benzoate formation from phenol and CO_2 in the presence of hydrogen

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Summary. Anaerobic phenol degrading consortia were selected in sewage sludge and culture conditions were improved to allow maximum degradation rates of 0.9 g/l·d. Phenol had to be added in two portions of 0.45 g/l at intervals of 12 h to keep the fermentation at stable conditions. From U-^{14}C-phenol little benzoate and acetate were formed as intermediates under a N_2:CO_2 gas phase. Final products were methane and CO_2. When methanogenesis was inhibited by BESA, less labeled methane and CO_2 were formed and labeled acetate remained undegraded. Turnover rates of phenol were significantly reduced in the presence of a H_2:CO_2 gas atmosphere and benzoate was formed from phenol and CO_2. Acetate did not accumulate remarkably. After the H_2:CO_2 was converted to methane or was exchanged by N_2:CO_2 the accumulated benzoate was further degraded to methane and CO_2. Elevated pools of acetate in sewage sludge led also to a reduction of the phenol degradation rates and presumably to an increased concentration of benzoate. In fresh sewage sludge benzoate degradation proceeds immediately, while the degradation of phenol starts only after a lag-phase of 3–10 days.

Introduction

Aromatic compounds exist in a variety of biogenic and abiogenic products, which are released into the environment. Many synthetic compounds are derived from phenol, of which more than 2.8 million tons were produced in 1979 (DFG-Bericht 1982).

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The degradation of aromatic compounds in nature by aerobic processes is known for a long time (e.g. Wagner 1914; Stanier 1950) and high removal rates in aerobic digesters for phenol have been reported recently (Ehrhardt and Rehm 1985; Bettmann and Rehm 1985). Aromatic compounds can also be degraded by anaerobic processes, e.g. by photometabolism (Dutton and Evans 1967), by nitrate-depending metabolism (Oshima 1965; Taylor et al. 1970; Taylor and Heeb 1972; Williams and Evans 1973, 1975), in sulfate-reducing environment (Widdel 1980) and under methanogenic conditions (Tarvin and Buswell 1934; Ferry and Wolfe 1976; Fina et al. 1978; Szewzyk et al. 1985; Tschech and Schink 1985). The anaerobic degradation of phenol with bacterial consortia has been reported by Chmielowski et al. (1965) and others (e.g. Healy and Young 1978). In pure culture trihydroxybenzene has been shown to be degradable (Schink and Pfennig 1982), while phenol can only be degraded in cocultures with methanogens or sulfate reducers (Mountfort and Bryant 1982; Mountfort et al. 1984; Barik et al. 1985). In this contribution we report on the selection and improvement of phenol-degrading consortia in sewage sludge and present for the first time evidence, that benzoate is formed from phenol, CO_2 and H_2 in sewage sludge.

Materials and methods

Source of phenol-degrading consortia and culture conditions

A phenol-degrading population was selected from a sewage sludge digester fed primary and secondary sewage sludge (1:1 mixture), obtained from the sewage treatment plant at Barbing, Regensburg. The sludge was fermented anaerobically at 37°C and at a hydraulic retention time (HRT) of 20 days in a stirred Biostat V reactor (Braun, Melsungen, FRG, total vo-

lume 2 l, working volume 1.5 l, 50 rpm). Once every day digested sludge was replaced manually by fresh sewage sludge to ensure the desired HRT. After reaching steady state conditions the fermenter content and the daily portion of fresh sludge were supplemented with 0.2 g phenol/l to select for phenol-degrading microorganisms. When the added amount of phenol could be degraded within 24 h, the phenol concentration in the fermenter was successively increased until residual phenol was found after 24 h of incubation. A further improvement of the degradation rate was obtained by adding lower amounts every 12 h.

A fermenter fed 0.45 g phenol/l·d provided a selected consortium of phenol degraders for further experiments. From this sludge 5, 20 or 500 ml portions in serum bottles of 20, 120 or in Schott flasks (Schott, Mainz, FRG) of 1000 ml volume were subcultured, using the anaerobic technique of Balch et al. (1979). N_2:CO_2 (80:20%) at 1 bar pressure served as a gas phase. In some experiments H_2:CO_2 (80:20%, 3 bar) was used as a gas phase. Phenol was added as indicated from anaerobic stock solutions at pH 7. The subcultures were supplemented with 2—5% sterile, fresh sewage sludge supernatant every 24 h. Samples for analyses were withdrawn with syringes flushed oxygen-free with nitrogen. All cultures were incubated at 37° C on a rotatory shaker at 100 rpm.

Analyses

Fermentation gases were quantified with a gas chromatograph (Packard Model 427), equipped with a thermal conductivity detector. For the separation a 2 m teflon column (inner diameter ⅛″) packed with Porapac N (Supelco Inc., Bellefonte, PE) was used and nitrogen at a flow rate of 15 ml/min served as carrier gas. The injector temperature was 150° C, the detector temperature (filaments) was 200° C. Volatile fatty acids were determined with a gas chromatograph (Packard Model 433), equipped with a flame ionization detector. Detector and injector temperature were 210° C, the oven temperature was 170° C at an isothermal mode. A 2 m teflon column (⅛″ inner diameter), packed with Chromosorb 101 (Sigma, Munich, FRG) with 30 ml N_2/min as carrier gas was used for separation. ^{14}C-labeled metabolites were determined using a gas proportional counter (Packard Model 894) connected with the gas chromatograph. The gas stream was splitted 9:1. Aromatic compounds were determined with a gas chromatograph (Packard Model 437), equipped with a flame ionization detector. Separation of compounds was in a 2 m glass column (5 mm inner diameter) packed with Silicon OV 17 (Serva, Heidelberg, FRG) using a temperature programme from 70—170° C (rise: 15° C/min). Less than 10% of the phenol added to sewage sludge samples was absorbed to sludge particles as judged from the ratio of supplemented to detected phenol.

An aromatic compound, which was later identified as benzoate was purified from 300 ml phenol-degrading sewage sludge supernatant (obtained by centrifugation) through concentration by vacuum evaporation, solubilization in CCl_3H/MeOH/petrol ether (6v:2v:3v) and column chromatography on Silica gel (Serva, Heidelberg). The combined benzoate fractions were evaporated to dryness, recrystallized several times in hot/cold water, filtered and dryed over $CaCl_2$.

The pure substance was identified as benzoate by:

1. Determination of its melting point and comparison with that of authentic benzoate.
2. Co-chromatography on Silica plates (Merck No. 5545) and determination of the R_f-values (=0.64).
3. IR spectroscopy (Beckman Accu Lab TM1) of compressed KBr/benzoate.
4. NMR spectroscopy (Variant-T-60) at 60 Megahertz in deuterated CCl_3D against tetramethyl silane.

The carbon balance for the degradation of uniformly labeled ^{14}C-phenol (U-^{14}C-phenol) was determined in the presence or absence of 8 μmol 2-bromoethanesulfonic acid (BESA)/ml in 5 ml portions of phenol-adapted sewage sludge, fed with 125 μl of fresh sewage sludge and 2.2—2.6 μmol/ml U-^{14}C-phenol (0.43 μCi/μmol in the absence or 0.8 μCi/μmol in the presence of BESA). The cultures were incubated at 37° C for 24 h on a rotatory shaker at 100 rpm.

For the demonstration of benzoate formation from phenol 20 ml of sewage sludge degrading 0.45 g phenol/l·d were supplied with either 114 μmol U-^{14}C-phenol and a H_2:CO_2 or N_2:CO_2 (80:20%, 3 bar) gas phase or with unlabeled phenol and $^{14}CO_2$ as indicated later on. Cultures were regassed every 12 hours and incubated at 37° C on a rotatory shaker for 24 h before analysis.

For the extraction of ^{14}C-benzoate, formed as an intermediate in low amounts during phenol degradation, 1 mg of non-labeled benzoate was dissolved in 20 ml of sewage sludge supernatant. After acidification the benzoic acid was extracted 3 times with 20 ml of diethyl ether. Then 2 ml of 0.5 N NaOH were added and the ether was evaporated. The residual sodium benzoate was resolved in 500 μl of distilled water and purified from contaminants by Silica gel thin layer chromatography. Benzoate was made visible by spraying Fluorescin or Cresol green as indicators.

Radioactivity was counted in a scintillation counter (Berthold Betascint BR5000), using Bray's scintillation cocktail (Bray, 1960).

Chemicals

U-^{14}C-phenol and ^{14}C-sodium bicarbonate were purchased from Amersham, Radiochemical Center, Braunschweig.

Phenol, benzoate, CCl_3H, methanol, petrol ether were of analytical grade and were obtained from Merck, Darmstadt, FRG. Silica gel plates 60 F_{254} (No 5545) were also obtained from Merck, Darmstadt.

Results

Selection of a phenol-degrading bacterial consortium from sewage sludge

Sewage sludge samples from different laboratory digesters were anaerobically transferred into serum bottles, supplemented with 0.2 g phenol per l and incubated at 37° C on a rotatory shaker at 100 rpm. Phenol was slowly degraded after a lag-phase of 3—10 days in subcultures derived from different digesters (e.g. Fig. 1). After phenol degradation started, its concentration was increased to 3.2 mmol/l at day 15 and to 4.5 mmol/l at day 26. The degradation rates of phenol increased with time, indicating an improvement of the phenol-degrading flora. Approximately 10 μmol methane were generated from 3.5 μmol of fermented phenol. When benzoate was added to the sludge cultures instead of phenol, its degradation began

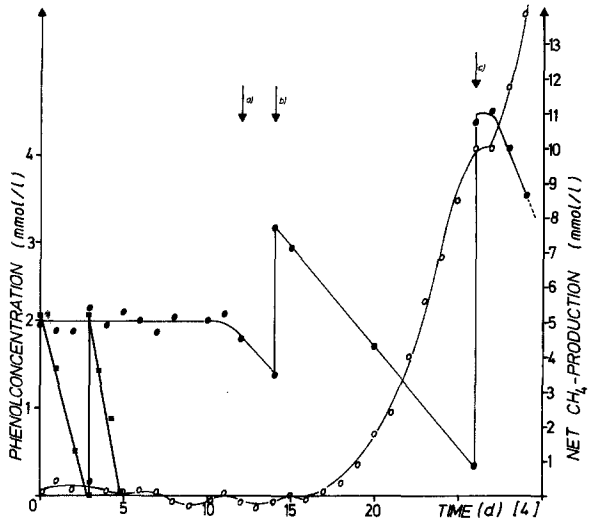

Fig. 1. Selection of a phenol- and benzoate-degrading consortium in sewage sludge. ●—● Phenol; ■—■ Benzoate; ○—○ Methane from phenol (corrected for methane production from sludge constituents in control cultures without phenol); ↓a) indicates the beginning of phenol degradation. At ↓b) the phenol concentration was increased to 3.2 mmol/l and at ↓c) to 4.5 mmol/l

immediately without a lag-phase (Fig. 1). Traces of oxygen were lethal for both, phenol- and benzoate-degrading consortia.

Improvement of phenol degradation in sewage sludge cultures

Phenol degradation rates were dependent on a concomitant supplementation with fresh sewage sludge or sewage sludge supernatant. 54% more phenol (4 versus 2.6 mmol/l·d) was degraded in cultures supplied with 50 ml sludge supernatant/l (≙ 20 d HRT) in addition to phenol than in cultures fed phenol only, indicating that some soluble sludge components may be limiting factors.

After selection of a phenol-degrading consortium in sewage sludge the phenol concentration was successively increased with each daily addition, provided that the previously supplied portion had been completely degraded. Phenol turnover rates and the methane production were measured (Fig. 2). Within one week only the turnover rates improved from 2.1 to more than 5 mmol/l·d at stable fermentation conditions. Phenol concentrations of up to 7.5 mmol/l·d could be degraded in 1 day, but the fermentation remained stable for only a few days and then failed. Up to 10 mmol

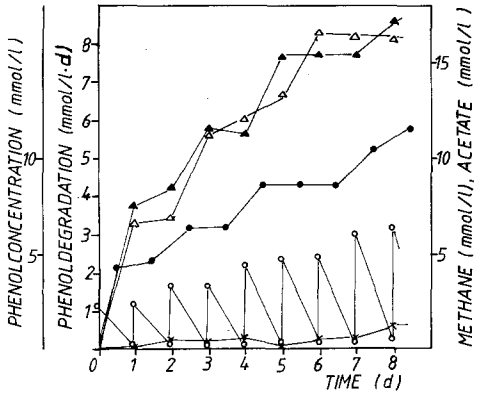

Fig. 2. Improvement of phenol degradation rates in sewage sludge. ×—× Acetate; ○—○ Phenol concentration; ●—● Phenol degradation rate; △—△ Methane generated; ▲—▲ Methane theoretically expected

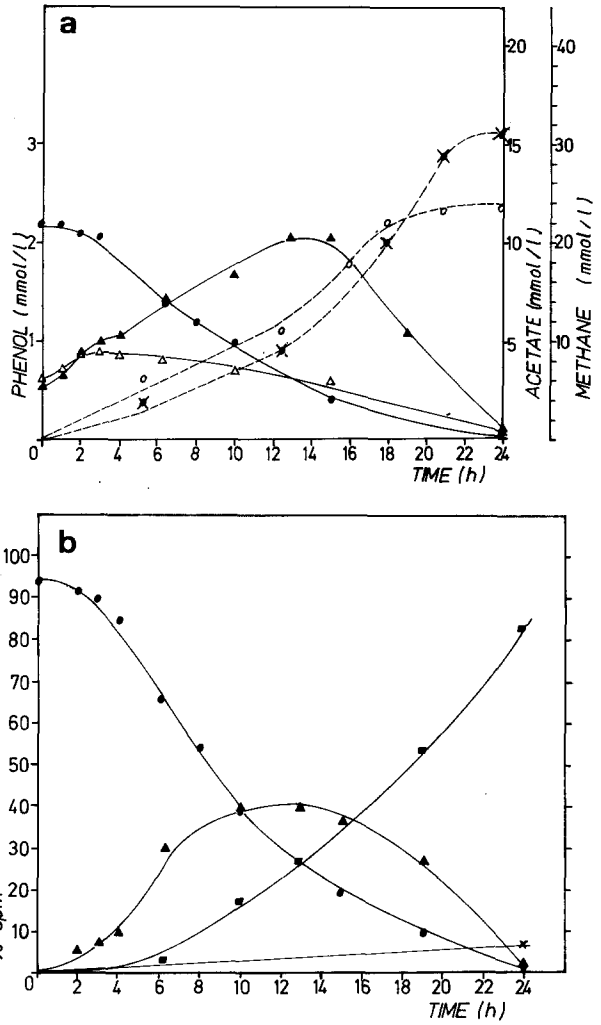

Fig. 3. Kinetics of U-^{14}C-phenol degradation and product formation. **a:** ●—● Phenol consumption; ▲—▲ Acetate production; △—△ Acetate production in sewage sludge without phenol; ×---× Methane production; ○---○ Methane production in control cultures without phenol. **b:** ●—● U-^{14}C-phenol; ▲—▲ ^{14}C-acetate; ■—■ $^{14}CO_2 + {}^{14}CH_4$; ×—× Activity in sludge pellet

Table 1. Carbon balance for the degradation of U-^{14}C-phenol in sewage sludge

Sewage sludge culture	Amount of U-^{14}C-phenol		Specific activity	Amount of ^{14}C-labeled fermentation products									Sludge pellet	Recovery
				Methane				CO_2			Acetate			
	µmol	cpm × 10^6	cpm/µmol C	total µmol	from phenol µmol	cpm × 10^6	spec. act. cpm/µmol CH_4	from phenol µmol	cpm × 10^6	spec. act. cpm/µmol CO_2	total µmol	cpm × 10^6	cpm × 10^6	% cpm
+ Phenol	11	10.1	153030	154	39	5.2	33766[a]	28	3.7	132140[b]	4.3	0.4	0.7	99
− Phenol	—	—	—	115	—	—	133330[b]	—	—	—	4.3	—	—	—

cpm/µmol C = cpm/µmol carbon atoms of phenol (6)

[a] Specific activity of the total methane

[b] Specific activity calculated for methane or CO_2 derived from phenol (determined by radio gas chromatography and substraction of methane and CO_2 of the sewage sludge inoculum

phenol/l·d could, however, be degraded per day when phenol was added in portions not exceeding 5 mmol/l (see Fig. 4 later on).

Degradation of U-^{14}C-phenol and carbon balance

The degradation of U-^{14}C-phenol in sewage sludge cultures and the formation of intermediates and fermentation products was measured for 24 h. The disappearence of phenol and the formation of products is shown in Fig. 3a, the distribution of the ^{14}C-labeling into products in Fig. 3b. When phenol was degraded, acetate was accumulated first. Later on it was completely cleaved to methane and presumably CO_2 (Fig. 3a). Similarly the radioactivity first appeared in acetate and later in methane and CO_2 (Fig. 3b).

The carbon balance is summarized in Table 1. From 11 µmol phenol 39 µmol methane, containing 50% of the radioactivity and 28 µmol CO_2, containing 35% of the radioactivity were produced. The residual acetate (4.3 µmol) contained 4% of the ^{14}C-label and 6.7% were found in the sludge pellet. Considering that 5% of the added ^{14}C-labeling was lost by sample withdrawal, the carbon recovery was 99%. During the degradation of U-^{14}C-phenol in sewage sludge cultures, when methanogenesis was inhibited by BESA, the main portion of the ^{14}C-activity of phenol was found in acetate and only little $^{14}CH_4$ and $^{14}CO_2$ were formed (Table 2). Whether other labeled intermediates accumulated was not tested in this experiment.

Evidence for the formation of benzoate from phenol and CO_2 in sewage sludge

In an attempt to increase the excretion of other intermediates than acetate into the culture liquid in phenol-degrading sludge consortia a feed-back inhibition was forced upon the culture by feeding the methanogens H_2:CO_2 (80:20%). In the presence of H_2:CO_2 phenol degradation rates decreased from 6 to 4 mmol/l·d, while under a N_2:CO_2 gas atmosphere — under otherwise similar conditions — up to 10 mmol phenol/l·d could be degraded (Fig. 4a). The phenol concentrations in cultures under N_2:CO_2 and under H_2:CO_2 are shown in Fig. 4b. Due to the faster degradation of phenol under a N_2:CO_2 gas phase and the microbial toxicity of phenol concentrations above 5 mmol/l cultures under N_2:CO_2 had to be supplied with phenol twice every day. The acetate pool in such cultures was 4 mmol/l (Fig. 4c),

Table 2. Degradation of U-^{14}C-phenol by adapted sewage sludge cultures in the presence of 2-bromoethanesulfonic acid, a specific inhibitor of the methanogens

^{14}C-Activity in phenol	^{14}C-Activity in products				Losses by sample withdrawal	Recovery	
	Acetate	CO_2	Methane	Sludge pellet			
cpm × 10^6	cpm × 10^6	cpm × 10^6	cpm × 10^6	cpm × 10^6	cpm × 10^6	cpm × 10^6	% initial cpm
23.4	12.5	2.4	0.6	1.9	5.0	22.4	96.5

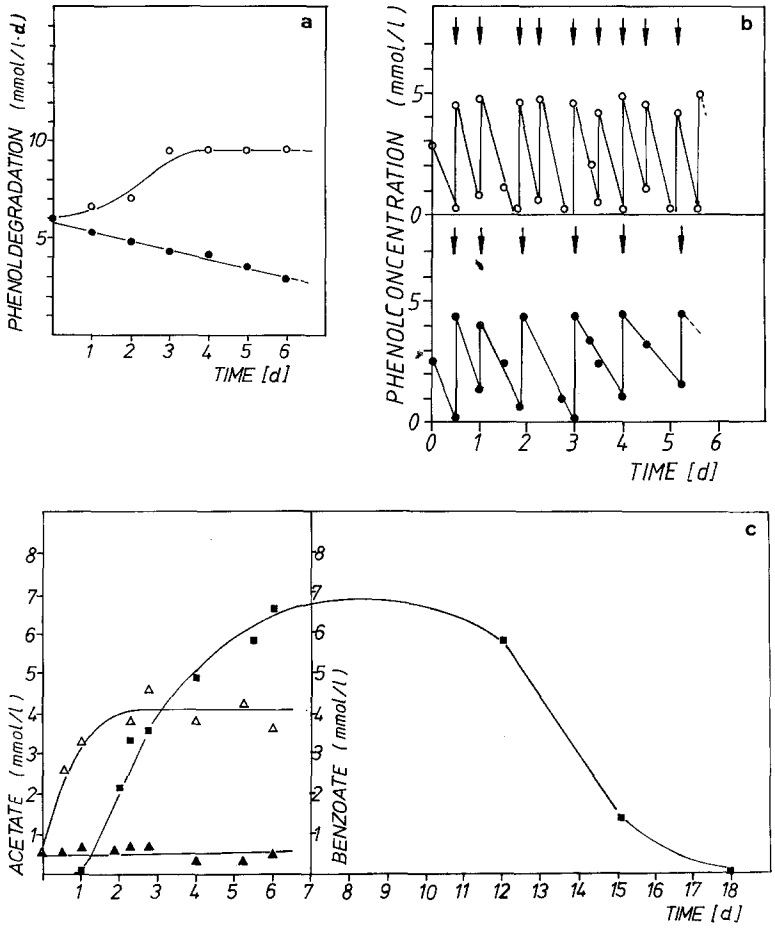

Fig. 4. Phenol metabolism by a sewage sludge consortium under a N_2:CO_2 (80:20%) or H_2:CO_2 (80:20%) gas atmosphere. **a:** Phenol degradation rates under N_2:CO_2 (○—○) or H_2:CO_2 (●—●). **b:** Phenol addition (↓) to cultures under N_2:CO_2 (○—○) or H_2:CO_2 (●—●) to concentrations as indicated. **c:** Acetate formation in cultures under a N_2:CO_2 gas atmosphere (△—△) and under a H_2:CO_2 gas atmosphere (▲—▲). Benzoate Formation (■—■) was only observed in cultures under a H_2:CO_2 gas atmosphere and was below the detection limit in cultures under a N_2:CO_2 gas atmosphere

while in cultures under H_2:CO_2 only little acetate was found. However, another metabolite, identified as benzoate, accumulated (Fig. 4c). The identification as benzoic acid was based on the melting point of the sodium salt (122° C versus 122.4° C for the authentic benzoate), on thin layer co-chromatography using Silica gel plates, on gas chromatographic features, on IR spectroscopy and on NMR spectroscopy, which revealed identical signals for the purified metabolite from phenol-degrading cultures and for authentic benzoate (not shown). The accumulated benzoate was further degraded to methane and CO_2 when the hydrogen was exhausted (Fig. 4c) or when it was replaced by N_2:CO_2.

Phenol degradation under a N_2:CO_2 gas phase could be suppressed by keeping the acetate concentration above 20 mmol/l (Fig. 5). However more methane was produced in these cultures, due to effective acetate cleavage. No benzoate accumulated to easily detectable quantities at acetate concentrations below 20 mmol/l.

^{14}C-labeled benzoate was formed from U-^{14}C-phenol or from non-labeled phenol and $^{14}CO_2$ in sewage sludge cultures in the presence of a H_2:CO_2 gas phase (Table 3). After 72 h of incubation 16.7% of the labeling of phenol was incorporated in benzoate in the presence of H_2:CO_2, while under N_2:CO_2 the phenol degradation to $^{14}CH_4$ and $^{14}CO_2$ was completed. However, some labeled benzoate, carrying 1.5% of the initially added activity of phenol could be extracted after 8 h of incubation, when phenol degradation was still proceeding at high rates (Table 3). Due to the dilution of $^{14}CO_2$ with CO_2 resolved in the sludge the benzoate pools were less labeled when unlabeled phenol and $^{14}CO_2$ were degraded (Table 3). However, more labeled benzoate was formed in the presence of H_2:CO_2 than in the presence of N_2:CO_2, which is consistent with the data obtained by feeding U-^{14}C-phenol and CO_2.

Discussion

Many aromatic compounds are toxic for microorganisms by interaction with the function of membranes (Eklund 1985). However, in recent years a

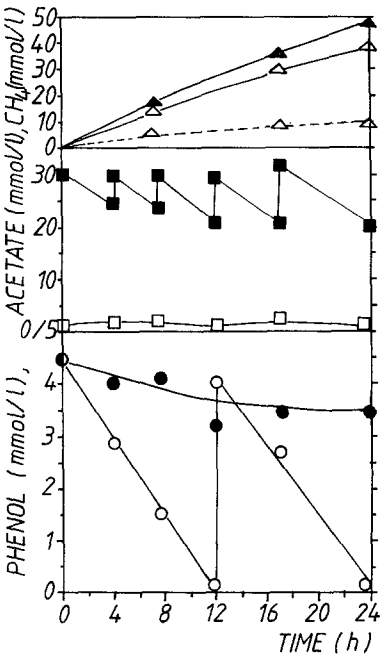

Fig. 5. Phenol metabolism by sewage sludge consortia at an artificially increased acetate pool. *Open symbols:* Acetate pool (□—□) not increased, ○—○ Phenol concentration and △—△ Methane production. Phenol addition after 12 h. *Closed symbols:* Acetate pool (■—■) artifically increased, ●—● Phenol concentration and ▲—▲ Methane production. △---△ Methane production in a control culture without phenol. ↑ Acetate addition

variety of complex consortia of different origin have been selected, which are able to degrade aromatic compounds at low concentrations (e.g. Ferry and Wolfe 1976; Healy and Young 1978; Szewzyk et al. 1985; Tschech and Schink 1985). As shown in this paper sewage sludge seems to contain reasonable numbers of benzoate-degrading microorganisms, while a phenol-degrading population must first be established, before phenol can be removed. High phenol removal rates of up to 1 g/l·d could be obtained when the phenol concentration did not exceed 0.5 g/l, requiring a periodic addition of phenol to serum flask cultures. Even higher phenol removal rates should be obtainable in chemostat cultures with a constant, optimal phenol supply (work in progress).

To further optimize the fermentation of phenol a detailed information about the phenol-degrading microorganisms, of their syntrophic relations to others and of the degradation pathway(s) is necessary. From thermodynamic considerations phenol can only be degraded in syntrophy with H_2 and acetate consuming organisms, according to equations 1 and 2 ($\Delta G_0'$ calculated after Thauer et al. 1977).

$$\text{Phenol} + 5\,H_2O \longrightarrow 3\,\text{Acetate} + 2\,H_2$$
$$\Delta G_0' = +5.6\ \text{kJ/mol} \qquad (1)$$

$$\text{Phenol} + 4\,H_2O \longrightarrow 3.5\,CH_4 + 2.5\,CO_2$$
$$\Delta G_0' = -167.12\ \text{kJ/mol} \qquad (2)$$

This was confirmed by reduced phenol degradation rates in experiments with elevated H_2 and acetate pools. Inhibitor experiments with BESA failed in this respect. This may be due to the complex sludge flora, containing also desulfuricants, which consume also hydrogen in competition with methanogens (Kristjansson et al. 1982).

The fact that benzoate accumulated in sewage sludge cultures when the phenol degradation was decreased by an artifical increase of end product pools and that benzoate was even found in low amounts in non inhibited cultures may indicate, that benzoate was either a regular intermediate of phenol degradation or that alternative pathways exists to those reported by Dutton and Evans (1967).

To our knowledge benzoate has not yet been shown to be a metabolite of anaerobic phenol degradation. The formation of benzoate from phenol and H_2/CO_2 or CO is energetically favourable

Table 3. Formation of benzoate from U-^{14}C-phenol and $^{14}CO_2$ during phenol degradation in sewage sludge cultures

Substrate/ Gas phase	Incubation time h	^{14}C-Activity added cpm × 10^6	Phenol added µmol/20 ml	Phenol degraded µmol/20 ml	^{14}C-Benzoate formed cpm × 10^6	% of ^{14}C added
U-^{14}C-Phenol + CO_2/ N_2:CO_2 (80:20%)	8	6.8	114	66	0.102	1.5
	72	6.8	228	228	0.001	0.0
U-^{14}C-phenol + CO_2/ H_2:CO_2 (80:20%)	72	4.6	228	169	0.780	16.7
Phenol + $^{14}CO_2$/ N_2:CO_2 (80:20%)	8	44.0	114	66	0.025	0.06
	72	44.0	228	228	0.000	0.00
Phenol + $^{14}CO_2$/ H_2:CO_2 (80:20%)	72	44.0	192	172	0.220	0.50

(Equations 3 and 4; $\Delta G'_0$-values calculated according to Thauer et al. 1977).

$$\text{Phenol} + CO_2 + H_2 \longrightarrow \text{Benzoate} + H_2O$$
$$\Delta G'_0 = -40.7 \text{ kJ/reaction} \quad (3)$$

$$\text{Phenol} + CO \longrightarrow \text{Benzoate}$$
$$\Delta G'_0 = -60.85 \text{ kJ/reaction} \quad (4)$$

Based on the different degradation pathways for phenol and benzoate proposed by Dutton and Evans (1967), a hypothetic scheme for the degradation of phenol by a methanogenic consortium is presented in Fig. 6. Evidence for the metabolism of phenol via benzoate is presented, but it remains to be elucidated whether the initial carboxylation proceeds with CO_2 and H_2 or with carbon monoxide.

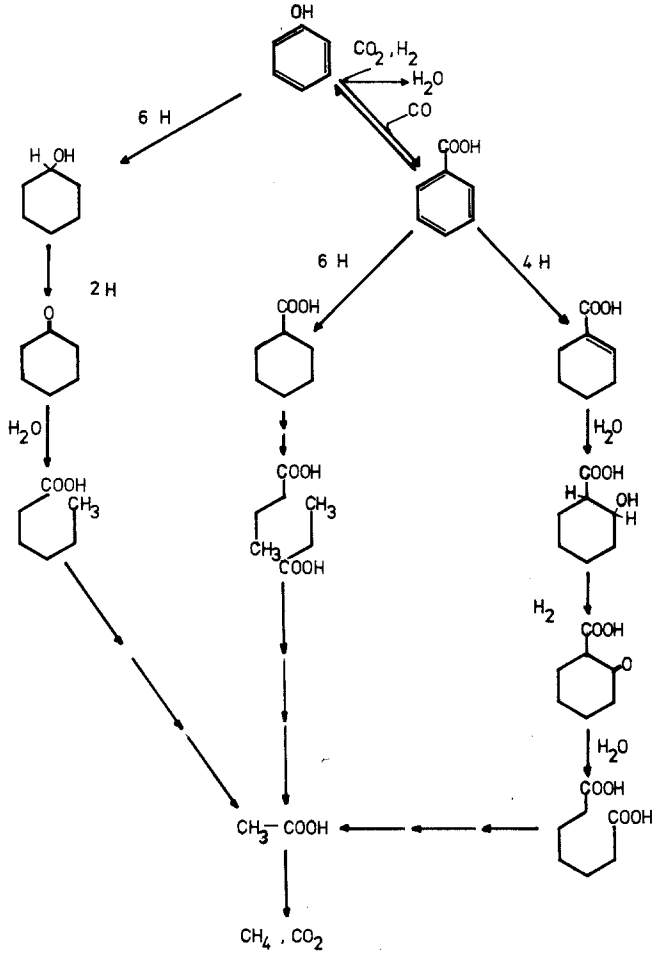

Fig. 6. Hypothetic scheme for the degradation of phenol by sewage sludge consortia

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References

Balch WE, Fox GE, Magrum LJ, Woese CR, Wolfe RS (1979) Methanogens: Reevaluation of a unique biological group. Microbiol Rev 43:260—296

Barik S, Brulla WJ, Bryant MP (1985) PA1, a versatile anaerobe obtained in pure culture, catabolizes benzoids and other compounds in syntrophy with hydrogenotrophs, and P-2 plus *Wolinella* sp. degrades benzoids. Appl Environ Microbiol 50:304—310

Bettmann H, Rehm HJ (1985) Degradation of phenol by polymer entrapped microorganisms. Appl Microbiol Biotechnol 20:285—290

Bray GA (1960) A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Anal Biochem 1:279—285

Chmielowski J, Grossmann A, Labazek S (1965) Biochemical degradation of some phenols during methane fermentation. Zesz Nauk Politech Slask Inz Sanit 8:97—122

DFG-Forschungsbericht (1982) Schadstoffe im Wasser, Vol. II Phenole. Deutsche Forschungsgemeinschaft (ed), D-5300 Bonn 2, Harald Boldt Verlag, Boppard

Dutton PH, Evans WC (1967) Dissimilation of aromatic substrates by *Rhodopseudomonas palustris*. Biochem J 104:30—31

Ehrhardt HM, Rehm HJ (1985) Phenol degradation by microorganisms adsorbed on activated carbon. Appl Microbiol Biotechnol 21:32—36

Eklund D (1985) The effect of sorbic acid and esters of p-hydroxybenzoic acid on the proton motive force in *Escherichia coli* membrane vesicles. J Gen Microbiol 131:73—76

Ferry JG, Wolfe RS (1976) Anaerobic degradation of benzoate to methane by a microbial consortium. Arch Microbiol 107:33—40

Fina LR, Bridges RL, Coblentz TH, Roberts FF (1978) The anaerobic decomposition of benzoic acid during methane fermentation. III. The fate of carbon four and the identification of propanoic acid. Arch Microbiol 118:169—172

Healy JB, Young LY (1978) Catechol and phenol degradation by a methanogenic population of bacteria. Appl Environ Microbiol 78:216—218

Kristjansson JK, Schönheit P, Thauer RK (1982) Different K_s-values for hydrogen of methanogenic and sulfate reducing bacteria: An explanation for the apparent inhibition of methanogenesis by sulfate. Arch Microbiol 131:278—282

Mountfort DO, Bryant MP (1982) Isolation and characterization of an anaerobic syntrophic benzoate-degrading bacterium from sewage sludge. Arch Microbiol 133:249—256

Mountfort DO, Brulla WJ, Krumholz LR, Bryant MP (1984) *Syntrophus buswelli* gen. nov. sp. nov.: A benzoate catabolizer from methanogenic ecosystems. Int J Syst Bact 34:216—217

Oshima T (1965) On the anaerobic metabolism of aromatic compounds in the presence of nitrate by soil microorganisms. Zeitschrift f. Allg. Mikrobiologie 5:386—394

Schink B, Pfennig N (1982) Fermentation of Trihydroxybenzenes by *Pelobacter acidigallici* gen. nov. sp. nov., a new strictly anaerobic non-sporforming bacterium. Arch Microbiol 133:195—201

Stanier RY (1950) Problems of bacterial oxidative metabolism. Bact Rev 14:179—191

Szewzyk M, Szewzyk R, Schink B (1985) Methanogenic degradation of hydroquinone and catechol via reductive dehydroxylation to phenol. FEMS Microbiol Ecol 31:79—87

Taylor BF, Campbell WL, Chinoy I (1970) Anaerobic degradation of the benzene nucleus by a facultatively anaerobic microorganism. J Bact 102:430—437

Taylor BF, Heeb MJ (1972) The anaerobic degradation of aromatic compounds by a denitrifying bacterium. Arch Microbiol 83:165—171

Tarvin D, Buswell AM (1934) The methane fermentation of organic acids and carbohydrates. J Am Chem Soc 56:1751—1755

Thauer RK, Jungermann K, Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. Bact Rev 41:100—180

Tschech A, Schink B (1985) Fermentative degradation of resorcinol and resorcylic acids. Arch Microbiol 143:52—59

Wagner R (1914) Über Benzolbakterien. Zschr Gärungsphysiol 4:289—319

Widdel F (1980) Anaerober Abbau von Fettsäuren und Benzoesäure durch neu isolierte Arten sulfatreduzierender Bakterien. Dissertation, Georg-August-Universität Göttingen, Göttingen

Williams RJ, Evans, WC (1973) Anaerobic metabolism of aromatic substrates by certain microorganisms. Biochem Soc Transactions 1:186—187

Williams RJ, Evans WC (1975) The metabolism of benzoate by *Moraxella* sp. through anaerobic nitrate respiration. Biochem J 148:1—10

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