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Energetics of product formation during anaerobic degradation of phthalate isomers and benzoate

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Abstract

Methanogenic enrichment cultures grown on phthalate, isophthalate and terephthalate were incubated with the corresponding phthalate isomer on which they were grown, and a mixture of benzoate and the phthalate isomer. All cultures were incubated with bromoethanosulfonate to inactivate the methanogens in the mixed culture. Thus, product formation during fermentation of the aromatic substrates could be studied. It was found that reduction equivalents generated during oxidation of the aromatic substrates to acetate were incorporated in benzoate under formation of carboxycyclohexane. During fermentation of the phthalate isomers, small amounts of benzoate were detected, suggesting that the initial step in the anaerobic degradation of the phthalate isomers is decarboxylation to benzoate. Gibbs free energy analyses indicated that during degradation of the phthalate isomers, benzoate, carboxycyclohexane, acetate and molecular hydrogen accumulated in such amounts that both the reduction and oxidation of benzoate yielded a constant and comparable amount of energy of approximately 30 kJ mol^{-1}. Based on these observations it is suggested that within narrow energetic limits, oxidation and reduction of benzoate may proceed simultaneously. Whether this is controlled by the Gibbs free energy change for carboxycyclohexane oxidation remains unclear. © 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Phthalate; Benzoate; Anaerobic; Energetics; Gibbs free energy

1. Introduction

The application of thermodynamic laws to biochemical processes provides a theoretical basis for analysis of experimental results. The second law of thermodynamics states that microbial conversions can only sustain growth if the reaction is exergonic. It further states that the amount of energy generated during a biochemical conversion equals the Gibbs free energy change of the chemical reaction. The best known application of thermodynamic principles for biotechnological processes is the observed correlation between the microbial yield and Gibbs free energy changes of microbial conversions [1–3]. Furthermore, the mechanisms of formation of intermediate compounds can be analysed using thermodynamic considerations [4–7]. A principal limitation of

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thermodynamic laws is that they provide no information about the rate of electron transport in complex microbial conversion reactions. It has been suggested, however, that Gibbs free energy dissipation values can be correlated with reaction rates in complex microbial conversions [8–10].

Microbial conversions in methanogenic environments proceed close to thermodynamic equilibrium. Several anaerobic fermentation reactions are endergonic under standard conditions. Examples of these reactions are the fermentation of ethanol, propionate, butyrate and benzoate to a mixture of acetate, carbon dioxide and hydrogen (or formate). Combined with the fact that methanogenic organisms only utilise a limited range of simple substrates (e.g. CO_2/H_2, formate, methanol or acetate), the degradation of substrates in methanogenic environments becomes dependent on a mixture of fermenting and methanogenic organisms. Only if the methanogenic organisms keep the concentration of the fermentation products low, the fermentation is exergonic and the reaction can be pulled to the product side. Because of their mutual dependence these mixed cultures are referred to as syntrophic cultures [11].

Concerning the minimum amount of Gibbs free energy needed to sustain growth and/or conversion of a substrate, some controversy exists. Values for critical Gibbs free energy changes for the fermentation of e.g. ethanol, propionate, butyrate, lactate or benzoate range from -2 to -30 kJ mol^{-1} [4,5,8,12–17]. Schink [11] postulated that the minimum amount of Gibbs free energy should equal approximately -20 to -30 kJ mol^{-1}, corresponding to 1/3 to 1/4 ATP. ATP can be synthesised in these small quanta via ion translocation across the cytoplasmic membrane. Considering, however, that anaerobic fermentations have been observed at Gibbs free energy changes much closer to 0 kJ mol^{-1}, it may be suggested that electron transport across the cytoplasmic membrane and ATP formation should be variable, implying that any exergonic reaction may sustain growth [18].

In previous studies we described the kinetics and related specific characteristics of three methanogenic enrichment cultures grown on phthalate, isophthalate and terephthalate [19,20]. It was postulated in these papers that the phthalate isomers are fermented to a mixture of acetate and hydrogen with benzoate (or benzoyl-CoA) as the central intermediate, analogous to the denitrifying organism *Pseudomonas* K136 [21,22]. All three cultures were found to have the ability to degrade benzoate without a lag phase, and they were only able to degrade the phthalate isomer on which they were cultivated. In this paper we describe product formation of the three cultures incubated with the phthalate isomer, or a mixture of benzoate and the phthalate isomer, in the presence of bromoethanosulfonate (BES), a specific inhibitor of methanogenesis. The Gibbs free energy changes of the observed conversions were calculated, and the implications for the energetic limits of the conversions are discussed.

2. Materials and methods

2.1. Biomass

Three enrichment cultures were used with the ability to degrade either phthalate, isophthalate or terephthalate. The cultures were obtained from digested sewage sludge or granular sludge and were enriched on one of the three phthalate isomers as described previously [19]. The phthalate isomer-grown cultures had the ability to degrade benzoate without a lag phase, at rates comparable to the phthalate isomers' degradation rates.

2.2. Experimental procedure

Experiments were performed in 117-ml serum bottles using a liquid volume of 25 or 40 ml. Medium, substrate and BES were added to the bottles from concentrated stock solutions. The composition of the medium has been described elsewhere [19]. Either the phthalate isomer or a mixture of the phthalate isomer and benzoate was used as substrate at concentrations between 2 and 5 mM. Serum bottles were sealed with butyl rubber stoppers and the headspace was flushed with an N_2/CO_2 mixture (70/30 v/v). Sulfide was dosed from a concentrated stock (final concentration 0.7 mM) to ensure anaerobic conditions. Serum bottles were pre-incubated at 37°C in an orbital shaker prior to inoculation with the phthalate isomer-grown cultures by syringe. Liquid samples (2 ml) were withdrawn from the bottles for

component analysis. All data shown are average values from duplicate experiments.

2.3. Analytical procedures

The concentration of aromatic acids was determined by high pressure liquid chromatography (HPLC). Intermediate formation of low concentrations of benzoate could be detected by injection of undiluted samples down to concentrations of 1 μM. The concentrations of acetate and carboxycyclohexane were measured by gas chromatography. The concentrations of hydrogen and methane in the headspace of the serum bottles were determined by gas chromatography as well. A full account of these analytical methods can be found elsewhere [19].

2.4. Energetic analyses

Standard Gibbs free energy changes for the observed conversions during degradation of benzoate and the phthalate isomers were calculated according to Thauer et al. [23] (Table 1). ΔG_f^0 values for the phthalate isomers were calculated from benzoate, using the group contribution method described by Dimroth [24]. The Gibbs free energy change for reduction of benzoate to carboxycyclohexane was calculated from the reduction of benzene to cyclohexane as suggested by Schink [11]. ΔG_f^0 values were corrected for a temperature of 37°C using the Van 't Hoff equation [25].

2.5. Mass and electron balances

Based on measured concentrations of all organic substrates and products, balances were derived to study whether non-identified products accumulated or analytical errors had occurred during the experiments. These balances are based on the concept of degree of reduction (γ, e-mol C-mol^{-1} or e-mol mol^{-1} for inorganic products) and the carbon chain length (Cl, C-mol mol^{-1}) of all relevant compounds. The degree of reduction of a compound is defined as the number of electrons liberated upon complete oxidation of 1 C-mol of organic material (or 1 mol of inorganic material) to CO_2 and H_2O [1]. Thus these balances are equivalent to chemical oxygen demand (COD)-based balances. Mathematically these balances can be described using the following equation:

$$\sum \gamma_S \cdot Cl_S \cdot C_S(t) + \sum \gamma_P \cdot Cl_P \cdot C_P(t) = \text{constant} \quad (1)$$

In this equation, C(t) stands for time-dependent concentration (M), and subscripts S and P stand for substrate(s) and product(s) respectively.

Another approach is based on the fact that during anaerobic fermentations, all electrons liberated during oxidation of a substrate should be incorporated in reduced products. Using the degree of reduction of the substrate as a frame of reference, the following electron balance can be derived:

$$\sum (\gamma_P - \gamma_S) \cdot Cl_P \cdot C_P\ (t) = 0 \quad (2)$$

Using this equation, oxidation is defined as negative and reduction as positive. If the product concentrations do not equal zero at the beginning of the experiment, the initial concentrations should be subtracted from the measured concentrations. From the stoichiometry of the fermentations shown in Table 1, it can be seen that inorganic carbon (HCO_3^-) is formed during the oxidation of benzoate and the

Table 1
Chemical reaction equations for the individual steps in mineralisation of phthalate isomers and benzoate, and standard Gibbs free energy changes during the conversions, corrected for a temperature of 37°C

Equation no.	Reaction	Equation	$\Delta G^{0\prime}$ (37°C) [kJ reaction^{-1}]
1	phthalate oxidation	$C_8H_4O_4^{2-}+8H_2O \rightarrow 3C_2H_3O_2^-+3H^++3H_2+2HCO_3^-$	38.9[a]
2	phthalate decarboxylation	$C_8H_4O_4^{2-}+H_2O \rightarrow C_7H_5O_2^-+HCO_3^-$	−20.7
3	benzoate oxidation	$C_7H_5O_2^-+7H_2O \rightarrow 3C_2H_3O_2^-+3H^+ +3H_2+HCO_3^-$	59.6
4	benzoate reduction	$C_7H_5O_2^-+3H_2 \rightarrow C_7H_{11}O_2^-$	−93.5
5	carboxycyclohexane oxidation	$C_7H_{11}O_2^-+7H_2O \rightarrow 3C_2H_3O_2^-+3H^++6H_2+HCO_3^-$	153.1

[a]Value represents calculated $\Delta G^{0\prime}$ for isophthalate and terephthalate fermentation, the value for *ortho*-phthalate fermentation is estimated to be 34.9 kJ reaction^{-1}. Applied $\Delta G_f^{0\prime}$ values for *ortho*-phthalate, isophthalate and terephthalate were −548.6, −552.6 and −552.7, respectively [24].

phthalate isomers. Oxidation equivalents formed due to HCO_3^- formation were calculated based on the measured acetate concentrations according to reaction stoichiometries shown in Table 1. Taking into account all conversions with benzoate shown in Table 1, the equation for benzoate fermentation becomes the following:

$$(\gamma_{C2}-\gamma_{BA})\cdot Cl_{C2}\cdot C_{C2} + (\gamma_{HCO_3}-\gamma_{BA})\cdot Cl_{HCO_3}\cdot \frac{C_{C2}}{3} + (\gamma_{CCH}-\gamma_{BA})\cdot Cl_{CCH}\cdot C_{CCH} + \gamma_{H_2}\cdot C_{H_2} = 0 \quad (3)$$

Subscripts C2, BA, HCO_3, CCH, and H_2, stand for acetate, benzoate, bicarbonate, carboxycyclohexane and molecular hydrogen respectively. Because decarboxylation of terephthalate to benzoate does not involve net oxidation or reduction, the same equation can be used for analyses of products formed during conversion of the phthalate isomers. Incorporation of electrons into biomass is ignored during these calculations.

3. Results

Phthalate-, isophthalate- and terephthalate-grown methanogenic cultures were incubated with the corresponding phthalate isomers, or a mixture of benzoate and the phthalate isomers in the presence of BES (20 mM). BES is a specific inhibitor of methanogenesis. During the incubation, substrate depletion and product formation were studied for analysis of product formation from the phthalate isomers and benzoate fermenting cultures.

It was found that during incubation of the isophthalate-grown culture with isophthalate as the sole carbon and energy source, benzoate, acetate, carboxycyclohexane and molecular hydrogen accumulated (Fig. 1). When the culture was incubated with a mixture of benzoate and isophthalate, benzoate was preferred over isophthalate, as was previously described for the terephthalate-grown enrichment culture [20]. As in the culture incubated with isophthalate, acetate, hydrogen and carboxycyclohexane accumulated during incubation with a benzoate/isophthalate mixture. In both experiments methane formation was negligible. Molecular hydrogen accumulated to comparable concentrations in both experiments, whereas acetate and carboxycyclohexane concentrations were slightly higher in the experiment incubated with a mixture of benzoate and isophthalate. Hydrogen concentrations were approximately 10 times higher than under exponential growth conditions [19].

It is evident from these data that part of the reducing equivalents generated during benzoate and isophthalate oxidation (reactions 1 and 3, Table 1) were incorporated into benzoate resulting in the formation of carboxycyclohexane (reaction 4, Table 1). From the electron balance, calculated according to Eq. 3 and shown in Fig. 1, it can be seen that the amount of reduction equivalents formed during oxidation of the aromatic substrates corresponds reasonably well to the amount of reduction equivalents incorporated in the reduced products. Furthermore, it should be noted that molecular hydrogen corresponds to less than 1% of the accumulating concentration of reduced products and therefore plays a minor role in the electron balance.

The actual Gibbs free energy changes of the different conversions observed were calculated from the measured substrate and product concentrations. Decarboxylation of isophthalate ($\Delta G' \cong -31$ kJ mol^{-1}, reaction 2, Table 1) and oxidation of isophthalate to acetate and hydrogen ($\Delta G' \cong -59$ kJ mol^{-1}, reaction 1, Table 1) remained exergonic throughout the period of the elevated hydrogen concentration. Gibbs free energy changes for oxidation and reduction of benzoate (reactions 3 and 4, Table 1) are shown in Fig. 1. In the experiment with isophthalate as sole substrate, both reduction and oxidation of benzoate remained exergonic throughout the experiment, and the Gibbs free energy change of approximately -30 kJ mol^{-1} for both conversions are highly comparable. Consequently, the Gibbs free energy change for oxidation of carboxycyclohexane remained around 0 kJ mol^{-1}, because the Gibbs free energy change for this conversion equals the value for benzoate oxidation minus the value for benzoate reduction. Also during the experiment with a mixture of isophthalate and benzoate, the Gibbs free energy change for carboxycyclohexane oxidation was relatively constant and close to 0 kJ mol^{-1}. The initial values for benzoate oxidation and reduction were, however, more negative than in the experiment with isophthalate as sole carbon source, due to the higher benzoate concentrations.

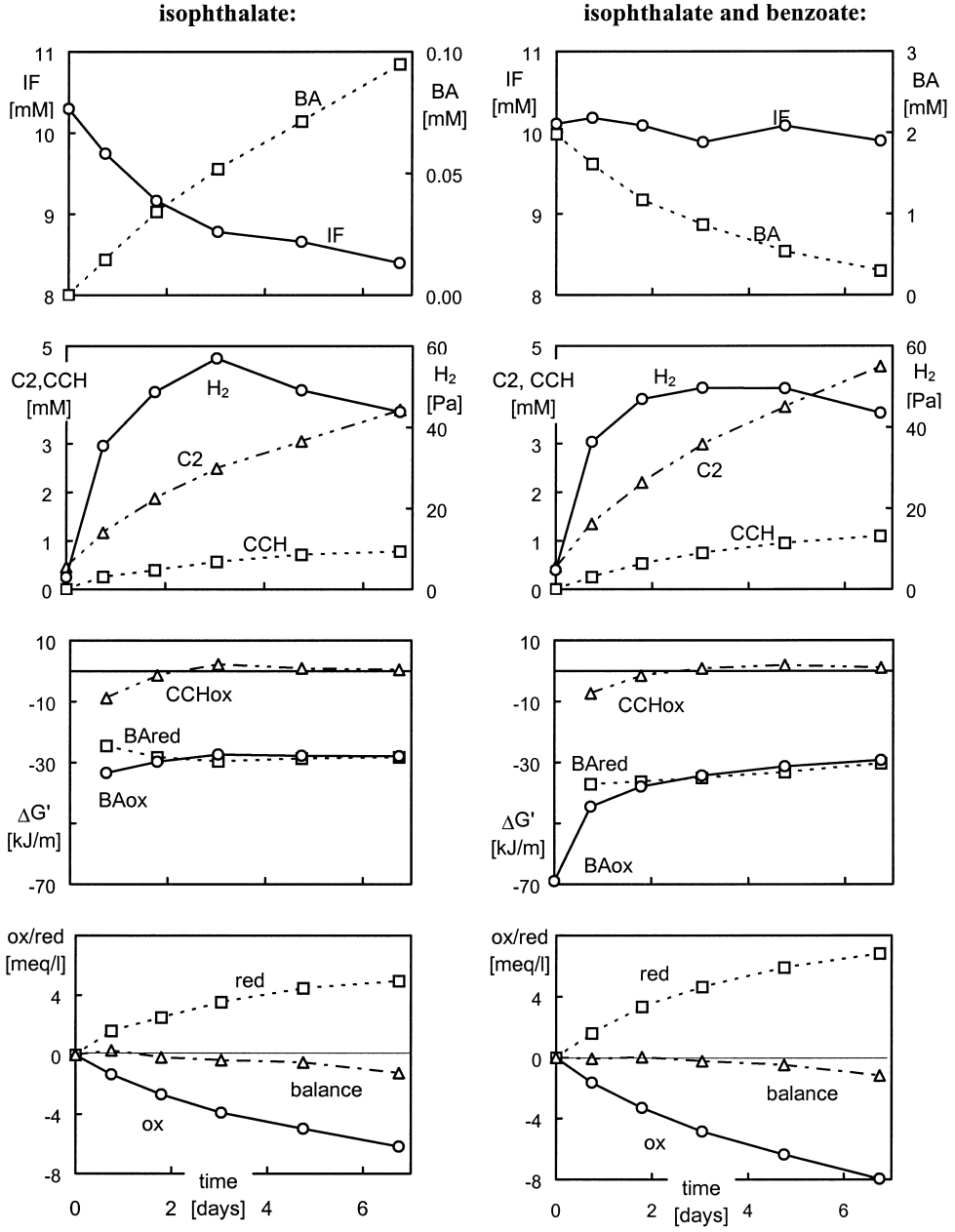

Fig. 1. Degradation of isophthalate (left graphs) and a mixture of isophthalate and benzoate (right graphs) by the isophthalate-grown enrichment culture in the presence of 20 mM BES. From top to bottom the graphs show (i) the concentration of isophthalate (IF) and benzoate (BA), (ii) the concentration of acetate (C2), carboxycyclohexane (CCH) and molecular hydrogen (H_2), (iii) the actual Gibbs free energy change for benzoate oxidation (BAox, reaction 3, Table 1), benzoate reduction (BAred, reaction 4, Table 1) and carboxycyclohexane oxidation (CCHox, reaction 5, Table 1), and (iv) the electron balance according to Eq. 2.

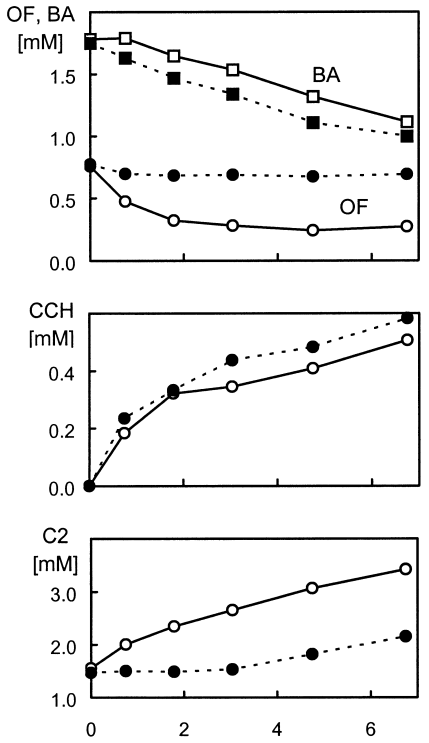

Fig. 2. Degradation of benzoate (BA, □/■) and phthalate (OF, ○/●, top graph) by the phthalate grown enrichment culture, and the accumulation of carboxycyclohexane (CCH, middle graph) and acetate (C2, bottom graph) in the absence (open markers, solid lines) and presence (solid markers, dashed lines) of hydrogen in the headspace.

The phthalate-grown culture was incubated with phthalate, and a mixture of phthalate and benzoate as well. In this case, experiments were also performed in the absence or presence of approximately 2.5% molecular hydrogen in the headspace of the serum bottles. Results of the experiments with a mixture of benzoate and phthalate are shown in Fig. 2, and the Gibbs free energy changes for the different conversions involving benzoate are shown in Fig. 3. In the absence of molecular hydrogen, benzoate and phthalate were initially converted to carboxycyclohexane and acetate. After approximately 3 days, phthalate conversion stopped and only benzoate was degraded further. As in the isophthalate experiment, the Gibbs free energy change for carboxycyclohexane oxidation was approximately 0 kJ mol^{-1}. In the presence of hydrogen in the headspace, no conversion of phthalate was observed, but benzoate was degraded at a comparable rate compared to the experiment without hydrogen. Initially, however, no acetate was produced, despite the observation that benzoate oxidation is exergonic, and all benzoate converted was reduced to carboxycyclohexane with molecular hydrogen. Acetate was only formed once the hydrogen concentration in the headspace had reached comparable values to those observed in experiments without hydrogen dosage. As with the bottles incubated with the benzoate/phthalate mixture, an initial reduction of phthalate to carboxycyclohexane was observed in serum bottles incubated with phthalate and molecular hydrogen (data not shown). Trace amounts of benzoate (approximately 50 μM) accumulated in these bottles. Once the Gibbs free energy change for carboxycyclohexane oxidation became exergonic due to hydrogen uptake, acetate formation was observed.

Product formation during degradation of phthalate and terephthalate by their corresponding enrichment cultures (without hydrogen dosage) showed a similar pattern as described for isophthalate. The

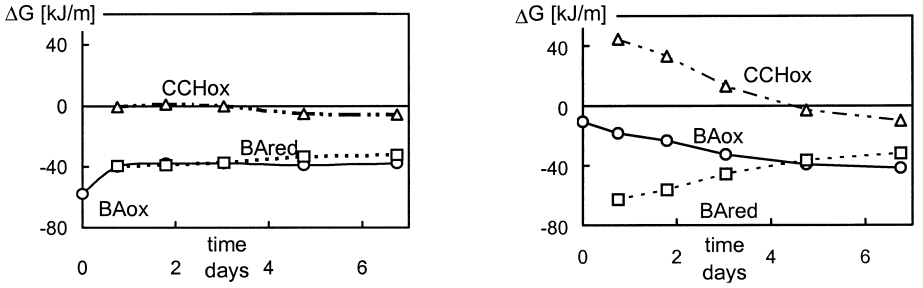

Fig. 3. Gibbs free energy changes for benzoate oxidation (BAox, ○), benzoate reduction (BAred, ■) and carboxycyclohexane oxidation (CCHox, △) in the absence (left graph) or presence (right graph) of molecular hydrogen in the headspace.

average values for the Gibbs free energy changes of the various conversions (Table 1) are presented in Table 2. It is evident that the Gibbs free energy changes of the different conversions were highly comparable for the different cultures utilised. A minor difference between the three cultures was that isophthalate degradation was completely inhibited by benzoate (Fig. 1), while limited conversion of phthalate and terephthalate was observed during incubation with benzoate (not shown). However, conversion of phthalate and terephthalate stopped within a few days when incubated with benzoate, even though benzoate conversion continued. This observation indicates that the cultures rapidly lost their capacity to convert the phthalate isomers in the presence of the preferred substrate benzoate, as described previously for the terephthalate-grown enrichment culture [20].

Because carboxycyclohexane was formed during our experiments, we checked the cultures for their ability to degrade carboxycyclohexane in the absence of BES. All three phthalate isomer-grown enrichment cultures were found to be capable of carboxycyclohexane degradation (initial concentration 5 mM) at rates ranging from 5 to 25% of the degradation rates of the phthalate isomers and without a lag phase (data not shown).

4. Discussion

The patterns of product formation during incubation with either the phthalate isomer or a mixture of phthalate and benzoate were found to be highly comparable for all three phthalate isomer-grown methanogenic enrichment cultures. In all experiments part of the phthalate isomer or benzoate was oxidised to acetate, reduction equivalents generated during the oxidation was incorporated in benzoate and carboxycyclohexane was formed. Small amounts of benzoate accumulated in the experiments incubated with the phthalate isomers as sole carbon and energy source. A schematic representation of the conversions observed is presented in Fig. 4.

Reduced product formation at elevated hydrogen partial pressures, comparable to the carboxycyclohexane formation in our experiments, has been observed before. Smith and McCarty demonstrated that ethanol perturbation of an ethanol- and propionate-fed chemostat led to accumulation of hydrogen in the biogas and consequently the formation of *n*-propanol and four to seven *n*-carboxylic acids [4,5]. Hickey and Switzenbaum suggested that hydrogen accumulated only to slightly higher concentrations during organic overloads of an anaerobic digester, due to reduced product formation [12,26].

The electron balances showed that 85–95% of the reduction equivalents generated during the oxidation of the aromatic substrates was incorporated into carboxycyclohexane. The observation that in all our experiments the concentration of reduced products could not fully account for the oxidised products detected suggests that an unidentified reduced product may have accumulated. A possible reduced product is formate. Formate has previously been identified as an alternative electron carrier for molecular hydrogen in methanogenic systems [11,27,28]. Mainly at elevated bicarbonate concentrations, as applied during our experiments, formate may play an important role in electron transfer among species. However, the calculated formate concentrations, considering a thermodynamic equilibrium between

Table 2
Actual Gibbs free energy change ($\Delta G'$, kJ reaction^{-1}) for the conversions shown in Table 1 in experiments incubated with the phthalate isomers as sole carbon and energy sources in the presence of BES

Culture	Phthalate isomer oxidation (reaction 1)	Benzoate oxidation (reaction 3)	Benzoate reduction (reaction 4)	Carboxycyclohexane oxidation (reaction 5)
Phthalate	−60.5 (1.3)	−32.5 (1.5)	−31.0 (1.5)	−1.5 (2.1)
Isophthalate	−59.6 (1.8)	−28.2 (1.1)	−28.7 (0.6)	0.5 (1.5)
Terephthalate	−62.2 (2.3)	−29.7 (2.8)	−27.4 (2.6)	−2.3 (1.6)

Calculations were performed during the time-period values were more or less constant and an equilibrium appeared to exist. Reaction numbers correspond to the chemical reaction equations shown in Table 1. Values between brackets represent standard deviations in the calculated $\Delta G'$ values.

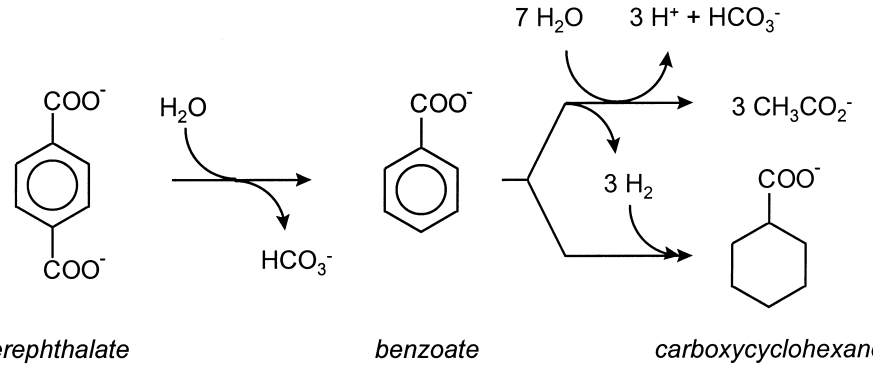

Fig. 4. Schematic representation of the conversions observed in the BES-amended phthalate isomer-grown cultures.

formate and hydrogen/bicarbonate, could not account for the observed gap in the electron balance (calculations not shown).

4.1. Energetics of the conversions observed

From the data in Table 2 it appears that all phthalate isomer-grown cultures gave comparable Gibbs free energy changes for all conversions observed. The energetics of the different conversions will therefore be discussed regardless of the phthalate isomer-grown culture utilised.

The Gibbs free energy change for oxidation of the phthalate isomers reached a stable value of approximately -60 kJ mol^{-1} in all cultures incubated with the phthalate isomer as sole carbon source. Even though initially some conversion of the phthalate isomers was observed in cultures incubated with a mixture of the phthalate isomers and benzoate, benzoate was the preferred substrate in all cases.

Both in cultures incubated with the phthalate isomers and the phthalate/benzoate mixture, the Gibbs free energy change for oxidation and reduction of benzoate was found to be around -30 kJ mol^{-1}. No benzoate conversion was observed at Gibbs free energy changes for oxidation or reduction of benzoate exceeding approximately -28 kJ mol^{-1}, suggesting that this value may represent a minimum amount of energy required to sustain the conversion of benzoate. This critical Gibbs free energy change for benzoate conversion is remarkably close to the value of -30 kJ mol^{-1}, reported by Warikoo et al. [15]. These authors determined the threshold concentrations of benzoate and the corresponding critical Gibbs free energy change as a function of the acetate concentration in a defined coculture consisting of the syntrophic benzoate degrader, strain SB, and the *Desulfovibrio* sp. strain G-11.

The observation that the Gibbs free energy changes for oxidation and reduction of benzoate are highly comparable in all our experiments suggests that the Gibbs free energy change for carboxycyclohexane oxidation is close to 0 kJ mol^{-1} (see Table 2). Two lines of reasoning can be used to describe the mechanistic basis for this observation: (i) carboxycyclohexane may be a true intermediate in the oxidation of benzoate, or (ii) it may be a side product originating from a different non-aromatic intermediate. The first possibility suggests that oxidation of benzoate only proceeds as long as the Gibbs free energy change for carboxycyclohexane oxidation is negative. The second possibility suggests that an equilibrium may develop if both oxidation and reduction of benzoate proceed at a comparable efficiency. In this case intermediates may accumulate to such concentrations that the Gibbs free energy changes for both oxidation and reduction of benzoate remain sufficiently negative to sustain growth and/or conversion of the aromatic substrate.

In summary, we suggest that within narrow energetic limits, simultaneous oxidation and reduction of benzoate may proceed. However, our experiments provide no answer to the question if carboxycyclohexane (or its CoA derivative) is an intermediate in the oxidation of benzoate, or a byproduct originat-

ing from a different non-aromatic intermediate. The fact that we worked with mixed cultures furthermore keeps the question open whether one or more organisms were involved in the conversions observed.

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References

[1] Heijnen, J.J., van Loosdrecht, M.C.M. and Tijhuis, L. (1992) A black box mathematical model to calculate auto- and heterotrophic biomass yields based on Gibbs energy dissipation. Biotechnol. Bioeng. 40, 1139–1154.

[2] McCarty, P.L. (1971) Energetics and bacterial growth. In: Organic Compounds in Aquatic Environments (Faust, S.D. and Hunter, J.V., Eds.), pp. 495–512. Marcel Dekker, New York.

[3] Stouthamer, A.H. (1979) The search for correlation between theoretical and experimental growth yields. Int. Rev. Biochem. 21, 1–47.

[4] Smith, D.P. and McCarty, P.L. (1989) Reduced product formation following perturbation of ethanol- and propionate-fed methanogenic CSTRs. Biotechnol. Bioeng. 34, 885–895.

[5] Smith, D.P. and McCarty, P.L. (1989) Energetic and rate effects on methanogenesis of ethanol and propionate in perturbed CSTRs. Biotechnol. Bioeng. 34, 39–54.

[6] Schink, B., Brune, A. and Schnell, S. (1992) Anaerobic degradation of aromatic compounds. In: Microbial Degradation of Natural Products (Winkelmann, G., Ed.), pp. 219–242, VCH, Weinheim.

[7] Wu, M.W. and Hickey, R.F. (1996) *n*-Propanol production during ethanol degradation using anaerobic granules. Water Res. 30, 1686–1694.

[8] Seitz, H.J., Schink, B. and Pfennig, N. (1990) Energetics of syntrophic ethanol oxidation in defined chemostat cocultures; energy requirements for H_2 production and H_2 oxidation (1). Arch. Microbiol. 155, 82–88.

[9] Seitz, H.J., Schink, B. and Pfennig, N. (1990) Energetics of syntrophic ethanol oxidation in defined chemostat cocultures; energy sharing in biomass production (2). Arch. Microbiol. 155, 89–93.

[10] Westerhoff, H.V., Lolkema, J.S., Otto, R. and Hellingwerf, K.J. (1982) Thermodynamics of growth; non-equilibrium thermodynamics of bacterial growth, the phenomenological and the mosaic approach. Biochim. Biophys. Acta 683, 181–220.

[11] Schink, B. (1992) Syntrophism among prokaryotes. In: The Prokaryotes (Balows, A., Trüper, H.G., Dworkin, M., Harder, W. and Schleifer, K.H., Eds.), Vol. I, pp. 276–299. Springer-Verlag, New York.

[12] Hickey, R.F. and Switzenbaum, M.S. (1991) Thermodynamics of volatile fatty acid accumulation in anaerobic digesters subject to increases in hydraulic and organic loading. Res. J. WPCF 63, 141–144.

[13] Dwyer, D.F., Weeg-Aerssens, E., Shelton, D.R. and Tiedje, J.M. (1988) Bioenergetic conditions of butyrate metabolism by a syntrophic anaerobic bacterium in coculture with hydrogen-oxidizing methanogenic and sulfidogenic bacteria. Appl. Environ. Microbiol. 54, 1354–1359.

[14] Seitz, H.J., Schink, B. and Conrad, R. (1988) Thermodynamics of hydrogen metabolism in methanogenic cocultures degrading ethanol or lactate. FEMS Microbiol. Lett. 55, 119–124.

[15] Warikoo, V., McInerney, M.J., Robinson, J.A. and Suflita, J.M. (1996) Interspecies acetate transfer influences the extent of anaerobic benzoate degradation by syntrophic consortia. Appl. Environ. Microbiol. 62, 26–32.

[16] Wallrabenstein, C. and Schink, B. (1994) Evidence of reversed electron transport in syntrophic butyrate or benzoate oxidation by *Syntrophomonas wolfei* and *Syntrophus buswellii*. Arch. Microbiol. 162, 136–142.

[17] Stams, A.J.M. (1994) Metabolic interactions between anaerobic bacteria in methanogenic environments. Antonie van Leeuwenhoek 66, 271–294.

[18] Thauer, R.K. and Morris, J.G. (1984) Metabolism of chemotrophic anaerobes: old views and new aspects. In: The Microbe 1984, Part II: Prokaryotes and Eukaryotes (Kelly, D.P. and Carr, N.G., Eds.), pp. 123–168. Cambridge University Press, Cambridge.

[19] Kleerebezem, R., Hulshoff Pol, L.W. and Lettinga, G. (1999) Anaerobic degradation of phthalate isomers by methanogenic consortia. Appl. Environ. Microbiol. 65, 1152–1160.

[20] Kleerebezem, R., Hulshoff Pol, L.W. and Lettinga, G. (1999) The role of benzoate in anaerobic degradation of terephthalate. Appl. Environ. Microbiol. 65, 1161–1167.

[21] Nozawa, T. and Maruyama, Y. (1988) Denitrification by a soil bacterium with phthalate and aromatic compounds as substrates. J. Bacteriol. 170, 2501–2505.

[22] Nozawa, T. and Maruyama, Y. (1988) Anaerobic metabolism of phthalate and other aromatic compounds by a denitrying bacterium. J. Bacteriol. 170, 5778–5784.

[23] Thauer, R.K., Jungermann, K. and Decker, K. (1977) Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. 41, 100–179.

[24] Dimroth, K. (1983) Thermochemische Daten organischer Verbindungen. In: D'ans-Lax, Taschenbuch für Chemiker und Physiker, Vol. 2, pp. 997–1038 Springer-Verlag, Berlin.

[25] Conrad, R. and Wetter, B. (1990) Influence of temperature on energetics of hydrogen metabolism in homoacetogenic, methanogenic, and other bacteria. Arch. Microbiol. 155, 94–98.

[26] Hickey, R.F. and Switzenbaum, M.S. (1991) The response and utility of hydrogen and carbon monoxide as process indicators

of anaerobic digestors subject to organic and hydraulic overloads. Res. J. WPCF 63, 129–140.

[27] Thiele, J.H. and Zeikus, J.G. (1988) Control of interspecies electron flow during anaerobic digestion: significance of formate transfer versus hydrogen transfer during syntrophic methanogenesis in flocs. Appl. Environ. Microbiol. 54, 20–29.

[28] Boone, D.R., Johnson, R.L. and Liu, Y. (1989) Diffusion of the interspecies electron carriers hydrogen and formate in methanogenic ecosystems and its implication in the measurement of K_m for hydrogen or formate uptake. Appl. Environ. Microbiol. 55, 1735–1741.