Anaerobic Degradation of Acetone and Higher Ketones via Carboxylation by Newly Isolated Denitrifying Bacteria

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Five strains of Gram-negative denitrifying bacteria that used various ketones as sole carbon and energy sources were isolated from activated sludge from a municipal sewage plant. Three strains are related to the genus *Pseudomonas*; two non-motile species have not yet been affiliated. All strains grew well with ketones and fatty acids (C_2 to C_7), but sugars were seldom utilized. The physiology of anaerobic acetone degradation was studied with strain BunN, which was originally enriched with butanone. Bicarbonate was essential for growth with acetone under anaerobic and aerobic conditions, but not if acetate or 3-hydroxybutyrate were used as substrates. An apparent K_s value of 5.6 mM-bicarbonate was determined for growth with acetone in batch culture. The molar growth yield was 24.8–29.8 g dry cell matter (mol acetone consumed)⁻¹, with nitrate as the electron acceptor in batch culture; it varied slightly with the extent of poly- β -hydroxybutyric acid (PHB) formation. During growth with acetone, $^{14}CO_2$ was incorporated mainly into the C-1 atom of the monomers of the storage polymer PHB. With 3-hydroxybutyrate as substrate, $^{14}CO_2$ incorporation into PHB was negligible. The results provide evidence that acetone is channelled into the intermediary metabolism of this strain via carboxylation to acetoacetate.

INTRODUCTION

Acetone enters natural environments from various sources. In anoxic environments, it can be formed by bacterial fermentations (Schardinger, 1905; Northrop *et al.*, 1919; Davies & Stephenson, 1941; Widdel, 1986) and it is also excreted by mammals during starvation or diabetes (Widmark, 1920). Bringmann & Kuhn (1977, 1978) reported that acetone has a weakly toxic effect on bacteria (MIC 530–1700 mg l⁻¹) and green algae (MIC 7500 mg l⁻¹) in comparison to other water pollutants. In aerobic waste water treatment, acetone is regarded as easily degradable (Roth, 1988).

Mechanisms of acetone degradation have been studied by several authors. In rats, two pathways of acetone degradation were found involving an initial oxygenation to acetol (for review, see Landau & Brunengraber, 1987) or a biotin-dependent carboxylation to acetoacetate (Plaut & Lardy, 1950). In aerobic bacteria, acetone is also degraded via oxygenation (Levine & Krampitz, 1952; Lukins & Foster, 1963; Vestal & Perry, 1969; Taylor *et al.*, 1980). In the absence of molecular oxygen, acetone is completely degraded to methane (Mazé, 1915). Evidence was provided recently that a carboxylation reaction plays a key role in this degradation (Platen & Schink, 1987). Also the denitrifying bacterium *Thiosphaera pantotropha* can degrade acetone (Robertson & Kuenen, 1983) and a carboxylation reaction is involved in this degradative process as well (Bonnet-Smits *et al.*, 1988). The same appears to be true for cell carbon synthesis from acetone by a non-oxygenic photosynthetic *Rhodocyclus gelatinosus*

Abbreviation: PHB, poly- β -hydroxybutyric acid.

(*Rhodospseudomonas gelatinosa*) strain (Siegel, 1950). A different mechanism of anaerobic acetone degradation has been suggested which involves hydration or phosphorylation to pronanediol or propanediol phosphate as intermediates (Rudney, 1954).

Here we describe newly isolated strains of denitrifying bacteria which degrade ketones in the absence of molecular oxygen. Evidence is provided that anaerobic growth with acetone depends on carbon dioxide, and that its degradation involves a carboxylation reaction as a primary step.

METHODS

Source of denitrifying ketone-degrading bacteria. The bacteria were enriched from activated sludge taken from the municipal sewage plant in Marburg, FRG.

Media. The carbonate-buffered mineral salts medium (modified after Widdel & Pfennig, 1981) used for enrichment cultures contained (in mmol l⁻¹): KH₂PO₄, 1.5; NH₄Cl, 3.7; Na₂SO₄·10H₂O, 0.5; NaCl, 17.0; MgCl₂·6H₂O, 2.0; KCl, 6.7; CaCl₂·2H₂O, 1.0. NaHCO₃ (30 ml l⁻¹ of a 1 M stock solution), trace element solution SL 10 (Widdel *et al.*, 1983; 1 ml l⁻¹), and vitamin solution (10-fold concentrated, after Widdel & Pfennig, 1981; 0.5 ml l⁻¹) were added after sterilization. The pH was adjusted to 7.2–7.4 with HCl. Rubber-stoppered serum bottles were half-filled with medium under a N₂/CO₂ (90:10, v/v) gas atmosphere.

The phosphate-buffered mineral salts medium contained (in mmol l⁻¹): NH₄Cl, 2.0; Na₂SO₄·10H₂O, 0.5; MgCl₂·6H₂O, 0.5; CaCl₂·2H₂O, 0.1; potassium phosphate buffer (pH 7.0), 3.0; sodium phosphate buffer (pH 7.0), 20.0; and trace element solution SL 10 (1 ml l⁻¹). The phosphate buffers (stock solution 0.5 M) and trace elements were added after autoclaving. To avoid contamination with carbon dioxide, the medium was cooled under an atmosphere of N₂ or Ar gas. Further additions (e.g. sodium nitrate, carbon sources) were made from 0.5–1.0 M stock solutions. Media were bottled in Hungate tubes or serum bottles under N₂ or Ar gas atmosphere.

TS-agar was prepared from Bacto tryptic soy broth (30 g l⁻¹, Difco) and agar (15 g l⁻¹).

Microbiological techniques. Pure cultures were obtained with the agar shake dilution technique (Pfennig & Trüper, 1981) using carbonate-buffered medium. Strain BunN was cultivated in Hungate tubes (17.5 ml volume) and infusion bottles of various volumes (100–1000 ml). Growth was determined by measuring turbidity in a Spectronic 20 photometer (Milton Roy Company, USA) at 440 nm in Hungate tubes. Standard identification techniques (oxidase, catalase, Gram-type, negative staining with Indian ink) were carried out as described by Süßmuth *et al.* (1987). Wet mounts for phase contrast photomicrographs were prepared after Pfennig & Wagener (1986). Tests for nitrogen fixation were carried out in liquid phosphate-buffered medium with 10 mM-3-hydroxybutyrate and without ammonia or nitrate salts, in either non-shaken or slightly shaken culture (70 r.p.m. in Erlenmeyer flasks without baffles). Autotrophic growth was tested in phosphate-buffered medium supplemented with 20 mM-sodium bicarbonate in 500 ml infusion bottles with 50 ml medium and a H₂/O₂ (80:20, v/v) gas atmosphere or 20 mM-sodium nitrate under 100% hydrogen. Growth with 20 mM-Na₂S₂O₃, 20 mM-NaNO₃ and 20 mM-sodium bicarbonate was tested in serum bottles under nitrogen gas atmosphere.

Carbonate- and substrate-free cell suspensions for inoculations were prepared by centrifugation (2600 g, 15 min) and washing twice in sterile phosphate-buffered medium. Dry weight was determined after washing cells twice in 20 mM-ammonium acetate solution and drying at 80 °C.

Chemical analyses of substrates and products. Acetone and propan-2-ol from culture broth were determined with a Carlo Erba Vega 6000 gas chromatograph with a 2 m × 2 mm column packed with 60/80 Carbowax C/0.3% Carbowax 20 M/0.1% H₃PO₄ (Supelchem, Sulzbach, FRG). The oven temperature was 80 °C, with the detector and injector at 200 °C; the carrier gas was N₂ with a flow rate of 45 ml min⁻¹. A 2 µl culture sample was injected. The retention time of acetone was 1.3 min and that of propan-2-ol, 2.0 min. Chromatograms were recorded with a Merck-Hitachi D-2000 integrator.

Nitrite was determined with sulphanilic acid and 1-naphthylamine as described by Procházková (1959). If nitrite was absent, nitrate was determined with chromotropic acid as described by Lange & Vejdeck (1980).

Analysis of cell compounds. Preparation of DNA and determination of guanine-plus-cytosine content was carried out after Mandel *et al.* (1970).

PHB was extracted from freeze-dried cells by shaking for 20–24 h in chloroform (25 ml chloroform per approx. 100 mg cells). The extract was filtered through a cellulose filter (type 5953, Schleicher & Schüll, FRG), the chloroform evaporated, and the dry residue dissolved in 1–4 ml chloroform. The PHB content of this extract was determined after conversion to crotonic acid (Law & Slepecky, 1961).

Incorporation of ¹⁴CO₂ into PHB was assayed by Schmidt degradation (Simon & Floss, 1967). PHB was converted to crotonic acid by heating to 100 °C in 95–97% H₂SO₄ for 15–20 min. The crotonate concentration was adjusted to 1.3–1.4 mM with 95–97% H₂SO₄. A 1 ml volume of this solution was adjusted to 100% H₂SO₄ by addition of 550 µl of fuming sulphuric acid (30% SO₃). Then 300 µl of this solution (containing about 260 nmol crotonic acid) was transferred into one part of the vessel of a Schmidt degradation apparatus (Fuchs *et al.*, 1980).

NaOH (1 M, 1 ml) was inserted into the other part of the vessel. After freezing at -70°C , 30 mg sodium azide was put on top of the solidified sulphuric acid solution. The reaction apparatus was closed, heated within 15 min to 70°C and kept at this temperature for a further 45 min. After cooling to room temperature, the carbon dioxide released from the crotonate was absorbed in NaOH over 5 h. For determination of radioactivity in the sulphuric acid fraction, 100 μl of the sample was neutralized by dropping into 900 μl ice-cold 3.9 M-NaOH. After acidification with formic acid, propionate was detectable in this preparation by gas chromatography without further oxidation.

Radioactivity was determined in a Rackbeta 'Spectral 1219' scintillation counter (LKB, Finland) after addition of 100–200 μl of neutral to alkaline (max. 1 M-NaOH) liquid sample to 20 ml of Quickszint 402 scintillation liquid (Zinsser, Frankfurt, FRG). D.p.m. were calculated after count rate correction for the disturbing effect of sodium azide and the quench effect of alkalinity.

All chemicals (analytical grade) were obtained from Fluka, FRG.

RESULTS

Enrichment of denitrifying bacteria with ketones

Anoxic non-reduced carbonate-buffered (30 mM) media (50 ml) with sodium nitrate (50 mM) and containing either acetone (8.2 mM), butanone (6.7 mM), pentan-2-one (5.6 mM), pentan-3-one (5.6 mM), hexan-2-one (4.9 mM), or hexan-3-one (4.9 mM) as the substrate were each

Table 1. *Properties of denitrifying bacteria isolated with various ketones*

All growth tests were carried out under anoxic conditions in carbonate- or phosphate-buffered medium with 10–25 mM-nitrate as electron acceptor, and, if not noted otherwise, 5 mM-substrate. All strains used acetate, propionate, butyrate, valerate, and caproate as substrates and did not grow with pentan-3-one, D(+) -galactose and L(–) -arabinose. +, Good growth; $\pm$, slow growth; –, no growth.

Character	Strain:				
	ActN	BunN*	Pon-2N	Hon-2N	Hon-3N
Motility	+†	–	+†	+†	–
Catalase	+	+	+	+	ND
Oxidase	+	+	+	+	ND
Optimal temp. for growth ($^{\circ}\text{C}$)	37	28	28	28	37
<i>Substrates</i>					
Acetone	+	+	+	–	–
Butanone	+	+	+	–	–
Pentan-2-one	–	+	+	–	+
Hexan-2-one	–	+	–	+	+
Hexan-3-one	–	–	–	–	+
Heptanoate	–	+	–	+	+
Isobutyrate	+	+	+	+	–
Isovalerate	+	+	+	+	–
Pyruvate	+	+	+	+	–
Citrate	–	–	ND	–	–
Succinate	+	+	+	+	–
Glycerol	–	+	–	–	–
D(+) -Glucose	–	–	+	–	–
D(–) -Fructose	–	–	+	–	–
D(+) -Xylose	ND	$\pm$	ND	ND	ND
Aerobic growth on TS-agar	+	+	+	+	–
G + C (mol%)	67.8	66.1	ND	66.5	66.3

ND, Not determined.

* BunN was further able to utilize: ethanol, propan-1-ol, propan-2-ol, butan-2-ol, crotonate, lactate, 3-hydroxybutyrate, malate, fumarate, aspartate, acetoacetyl ethyl ester (3.4 mM, slow growth). The strain did not utilize: 1,2-propanediol, 1,3-propanediol, 1,2-butanediol, 2,3-butanediol, 1,3-butanediol, benzoate (2.5 mM), glycinate, formate (sole carbon and energy source), hydroxyacetone (acetol), acetoacetamide, acetoacetone, acetoin, diacetyl (1.0 mM).

† Monotrichous monopolar.

inoculated with 5 ml of activated sludge. After 5 d incubation at 28 °C, gas formation and increased turbidity was observed in all cultures; in the enrichment culture with hexan-3-one, this happened only after 10 d. Samples (5 ml) of each of these cultures were transferred to 50 ml of fresh medium. After a further 4–10 d, bacteria were isolated from these subcultures by agar shake dilutions in the same medium containing the initial enrichment substrate. Colonies developed within 11 d and were purified in a second dilution series. Pure cultures of bacteria were obtained with acetone (strain ActN), butanone (strain BunN), pentan-2-one (strain Pon-2N), hexan-2-one (strain Hon-2N) and hexan-3-one (strain Hon-3N). All strains were Gram-negative rods. With pentan-3-one, only a very slow-growing enrichment culture was obtained which was not purified. Table 1 shows the characteristics of the strains isolated. Strain BunN was chosen for further study of acetone degradation because it grew well suspended in liquid culture, whereas the other strains ActN and Pon-2N formed flocs and thus prevented photometric growth measurement.

Characterization of strain BunN

Cells of strain BunN were straight to slightly curved rods ($1.0 \times 2.2 \mu\text{m}$), Gram-negative, non-motile, and formed inclusions of PHB (Fig. 1). Formation of a slime capsule was not observed on agar plates or in liquid media. After aerobic growth on TS-agar, catalase and oxidase were detected. No vitamins were needed in the phosphate-buffered mineral salts medium. In ammonium-free media, nitrate was used as the nitrogen source. Molecular N_2 was not fixed in ammonium or nitrate-free media supplemented with $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ or $\text{VOSO}_4 \cdot 5\text{H}_2\text{O}$ (each 5 mg l^{-1}). Under these conditions, with 3-hydroxybutyrate as substrate, PHB was formed at a high rate, rendering the cells highly refractile. Chemolithoautotrophic growth with carbon dioxide as the sole carbon source and molecular H_2 or thiosulphate as electron donors was not observed. Growth was optimal at 28 °C (slower growth at 37 °C, no growth at 42 °C). Growth occurred at pH values from 6.2 to 8.7; no growth was detectable at pH 5, and pH values higher than 8.7 were not tested. The guanosine-plus-cytosine content of the DNA was 66.1 mol% ($\pm 0.1 \text{ mol}\%$) as determined by thermal denaturation. Substrates tested as carbon sources are listed in Table 1. Strain BunN preferentially used ketones and carbonic acids as substrates. Xylose was the only sugar utilized, but growth was extremely slow. No growth occurred with acetol or 1,2-propanediol. Acetone, 3-hydroxybutyrate, valerate, and pyruvate were also degraded under aerobic conditions; other substrates were not tested aerobically.

Growth curve and stoichiometry of acetone degradation

A typical growth curve of strain BunN in phosphate-buffered mineral salts medium with 20 mM-bicarbonate, excess acetone and a limiting amount of nitrate is shown in Fig. 2. Nitrate was converted to nitrogen without measurable release of nitrite. Nitrite was released only in acetone-limited cultures (data not shown). Small amounts of propan-2-ol (0.2–0.3 mM) were formed if nitrate limited growth. The doubling time under optimal growth conditions was 5.7–6.0 h (Figs 2 and 3).

Acetone was degraded completely to CO_2 and water (Table 2). The molar growth yield was calculated from total cell dry matter (including PHB) and was 24.8 g mol^{-1} (culture no. 1) and 29.8 g mol^{-1} (culture no. 2).

Dependence of acetone degradation on carbon dioxide

Growth curves of strain BunN with acetone and increasing amounts of bicarbonate are shown in Fig. 3(a). No growth occurred if the medium lacked bicarbonate. Increasing amounts of bicarbonate led to an increase in growth rates. If bicarbonate was limiting, a tendency to biphasic growth was observed (Fig. 3a), especially at low bicarbonate concentrations. The initial doubling time was proportional to the reciprocal value of bicarbonate concentration in the medium; an apparent K_s for bicarbonate in batch culture was calculated to be 5.6 mM (Fig. 3b). Growth with acetate or 3-hydroxybutyrate did not depend on bicarbonate. The doubling times with these substrates were 4.8 h and 4.0 h, respectively.

Fig. 1

Fig. 1. Phase contrast photomicrograph of strain BunN. Bar, 10 μm . PHB appears as dark inclusions due to the preparation techniques applied.

Fig. 2

Fig. 2. Growth (OD_{440} , $\square$) of strain BunN with acetone and limiting amounts of nitrate. The experiment was carried out in Hungate tubes (17.5 ml) with 10 ml of phosphate-buffered medium in the presence of 20 mM-sodium bicarbonate. Nitrite was not detectable. $\circ$, Acetone; $\bullet$, propan-2-ol; $\triangle$, nitrate.

Table 2. *Electron recovery after growth of strain BunN with acetone and nitrate*

Two cultures of 945 ml and 980 ml volume with initially 10 mM-acetone and 10.2 mM (± 0.03 mM) sodium nitrate were inoculated with washed cells. The initial turbidity measured in Hungate tubes at 440 nm was 0.072 (culture no. 1) and 0.080 (culture no. 2). The dry weight calibration value was 21.5 mg l^{-1} at $\text{OD}_{440} = 0.1$. Cells were harvested in the exponential growth phase.

	Culture no. 1		Culture no. 2	
	Measured parameter	Stoichiometric amount of acetone used (mmol)	Measured parameter	Stoichiometric amount of acetone used (mmol)
Acetone-consuming reaction*				
Nitrate consumed ¹	9.65 mmol	3.02	8.99 mmol	2.81
Propan-2-ol formed ²	0.38 mmol	0.43	0.31 mmol	0.35
Cell material formed ³ (not including PHB)	109.9 mg	1.13	93.3 mg	0.96
PHB formed ⁴	0.8 mg	0.01	41.2 mg	0.54
Acetone consumption calculated from formed products		4.59		4.66
Acetone consumed (measured)		4.90		4.87
Electron recovery		93.7%		95.7%

* Amounts of acetone consumed were calculated by the following reaction equations:

- (1) $5 \text{ C}_3\text{H}_6\text{O} + 16 \text{ NO}_3^- + 16 \text{ H}^+ \rightarrow 15 \text{ CO}_2 + 8 \text{ N}_2 + 23 \text{ H}_2\text{O}$
- (2) $9 \text{ C}_3\text{H}_6\text{O} + 5 \text{ H}_2\text{O} \rightarrow 8 \text{ C}_3\text{H}_8\text{O} + 3 \text{ CO}_2$
- (3) $17 \text{ C}_3\text{H}_6\text{O} + 13 \text{ CO}_2 + 5 \text{ H}_2\text{O} \rightarrow 16 (\text{C}_4\text{H}_7\text{O}_3)$
- (4) $9 \text{ C}_3\text{H}_6\text{O} + 5 \text{ CO}_2 \rightarrow 8 (\text{C}_4\text{H}_6\text{O}_2) + 3 \text{ H}_2\text{O}$

Fig. 3. Dependence of growth of strain BunN with acetone (5 mM) and sodium nitrate (5 mM) on the initial sodium bicarbonate concentration in the medium. Experiments were done in Hungate tubes with 10 ml medium under N₂ (CO₂ free). (a) Semi-logarithmic plot of growth curves (OD₄₄₀) with sodium bicarbonate concentrations of 1.0 mM (■), 2.5 mM (△), 5.0 mM (▲), 10.0 mM (○), and 20.0 mM (●). The control culture without bicarbonate did not grow (not shown). Bar equals $\Delta \log \text{OD}_{440} = 0.05$. Initial OD was 0.08 in all cases. (b) Plot of initial doubling times against the reciprocal of bicarbonate concentration. Initial doubling times were taken from (a).

Table 3. Distribution of ¹⁴CO₂-label in crotonate prepared from PHB from cells of strain BunN grown with acetone and ¹⁴CO₂-labelled bicarbonate

The experimental conditions are described in the text. Deviations refer to four or five independent measurements of the same preparation.

Preparation before Schmidt degradation	Preparation after Schmidt degradation	Radioactivity released as CO ₂	Recovery of radioactivity
264 nmol crotonate 3553 d.p.m. (± 3%)	18 nmol crotonate 934 d.p.m. (± 12%)	2285 d.p.m. (± 5%)	3219 d.p.m. (90%)

Under aerobic conditions, acetone degradation also depended on bicarbonate. The CO₂ content of air (0.03%) was too low for growth with acetone; however, the culture grew well under these conditions if 20 mM-sodium bicarbonate was added in closed culture bottles under air.

Incorporation of ¹⁴CO₂ into PHB

A dense cell suspension (OD₄₄₀ 14.9, corresponding to 80–85 mg dry cells in 27 ml) was incubated in phosphate-buffered medium containing 15 mM-acetone, 50 mM-sodium nitrate, 15 mM-sodium bicarbonate with a specific radioactivity of 1.1×10^8 d.p.m. mmol⁻¹, and no ammonium salts. After 4.75 h of incubation at 28 °C, all acetone was consumed. From 73.5 mg freeze-dried cells, 30.4 mg lipid was extracted containing 28.3 mg PHB (93%). The specific radioactivity of PHB was $1.54 \pm 0.15 \times 10^5$ d.p.m. (mg PHB)⁻¹ [corresponding to $1.33 \pm 0.13 \times 10^7$ d.p.m. (mmol crotonate)⁻¹]. Schmidt degradation of crotonate showed that most (71%) of the labelled carbon was released as CO₂; 29% of the radioactivity remained in the residual lipid fraction (Table 3).

If 3-hydroxybutyrate was used as substrate in the suspension experiment, the specific radioactivity of the PHB formed was only 6.4% of that obtained with acetone as substrate.

DISCUSSION

Enrichment cultures with various ketones in anoxic carbonate-buffered medium led to the isolation of five strains of aerobic, facultatively denitrifying bacteria from activated sludge. Contrary to nearly all ketone-degrading isolates described so far (Levine & Krampitz, 1952;

Lukins & Foster, 1963; Vestal & Perry, 1969; Taylor *et al.*, 1980; Coleman & Perry, 1984), all these new isolates were Gram-negative. The only Gram-negative bacterium degrading acetone both aerobically and under denitrifying conditions is *Thiosphaera pantotropha* (Robertson & Kuenen, 1983).

The taxonomic affiliation of our new isolates can so far only partially be defined. They all have to be grouped with the Gram-negative aerobic rods in section 4 of *Bergey's Manual of Systematic Bacteriology* (Krieg, 1984). Strains ActN, Pon-2N and Hon-2N belong to the *Pseudomonadaceae*, due to their polar monotrichous flagellation and positive catalase and oxidase reaction. Strain Pon-2N grows with sugars as do most members of the *Pseudomonadaceae*, whereas strains ActN and Hon-2N do not use sugars, which is similar to *Pseudomonas alcaligenes* and *P. diminuta* (Palleroni, 1984). The non-motile strains Hon-3N and BunN share several properties with the *Azotobacteraceae* and *Rhizobiaceae*, as well as with the genera *Beijerinckia* and *Derxia* (2-propanol selectively enriches for strains of these genera; Becking, 1984); however, they are unable to fix nitrogen. Failure to grow autotrophically indicates that BunN is not related to *Thiosphaera pantotropha* (Robertson & Kuenen, 1983). Further studies will be necessary to allow a definitive taxonomic affiliation of these isolates.

The physiology of nitrate-dependent acetone degradation was studied in detail with strain BunN. It degraded acetone completely to CO₂. Small amounts of propan-2-ol were formed in nitrate-limited cultures, possibly by an propan-2-ol dehydrogenase which also enables this strain to use propan-2-ol as a substrate.

CO₂ was present at about 30 mM in the carbonate-buffered medium used for all enrichments, and it turned out that CO₂ was essential for good growth with acetone as the substrate. In contrast, growth with acetate or 3-hydroxybutyrate was not CO₂-dependent. Anaerobic acetone degradation by our isolate, BunN, involves a primary carboxylation reaction, as also demonstrated by the labelling experiments with ¹⁴CO₂, in which 71% of the incorporated CO₂ was found in the C-1 atom of PHB hydrolysis products, indicating that acetone is first carboxylated to acetoacetate. Twenty-nine percent of the label was not released as CO₂ in Schmidt degradation. This finding may be due to two reasons: (1) acetoacetyl-CoA and acetyl-CoA are in equilibrium with each other (Senior & Dawes, 1973) and may actively exchange during our incubation experiment thus also labelling the C-3 atom of 3-hydroxybutyrate, and (2) the labelled acetoacetyl-CoA is a precursor of other lipids and is also incorporated into cellular compounds during the incubation time. With these results and the fact that ¹⁴CO₂ was incorporated into PHB only to a small extent with 3-hydroxybutyrate as the substrate it appears, therefore, that strain BunN degrades acetone via carboxylation with CO₂ to an acetoacetate residue. Thus an initial hydration as suggested by Rudney (1954) can be excluded; strain BunN is unable to grow with 1,2-propanediol which would be the first intermediate of such a degradation pathway.

Nitrate-dependent acetone oxidation by strain BunN appears to take a similar initial reaction sequence via carboxylation as first described for the phototroph *Rhodocyclus gelatinosus* (*Rhodopseudomonas gelatinosa*; Siegel, 1950) and recently for a methanogenic enrichment culture (Platen & Schink, 1987). Also *Thiosphaera pantotropha* degrades acetone via carboxylation (Bonnet-Smith *et al.*, 1988). These bacteria were apparently all enriched and isolated in media containing bicarbonate and CO₂ at enhanced concentrations (30 or 44 mM). These concentrations are well suited to satisfy acetone carboxylating enzyme systems with CO₂ affinities similar to that of our strain BunN (apparent *K_s* 5.6 mM for bicarbonate). Aerobic acetone degraders, on the other hand, were always enriched in media in which the CO₂ content was in equilibrium with air, namely, about 63 µM (calculated after Stumm & Morgan, 1981). It is not surprising, therefore, that all these isolates appear to degrade acetone via an oxygenase reaction rather than via carboxylation. Although oxygenase-catalysed acetone degradation is far less energy-efficient than activation via carboxylation, it may be advantageous under conditions of low substrate supply as this is probably typical of oxic natural habitats.

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