Cultural Factors Influencing the Utilization or Production of Acetate by *Butyrivibrio fibrisolvens*

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SUMMARY

Utilization of acetate by four strains of *Butyrivibrio fibrisolvens* was influenced by the composition of their growth medium. Growth-limiting glucose concentrations, low availability of CO₂ and the presence of sodium lactate all stimulated acetate uptake by three strains. The type strain, D₁, utilized acetate if the concentration of acetate added to the medium was at least 15 µmol ml⁻¹. In batch culture, all strains produced acetate before entering a phase of acetate uptake. Continuous-culture studies with one strain showed that acetate uptake was dependent upon growth rate. The amount of n-butyrate produced in batch or continuous culture was closely linked to the amount of acetate taken up.

INTRODUCTION

Bacteria belonging to the genus *Butyrivibrio* produce large amounts of n-butyric acid by fermentation of carbohydrates (Bryant & Small, 1956). They generally occur as a major part of the microflora in the rumen of animals given high roughage rations and having a rumen fermentation characterized by a large molar proportion of acetic acid (Shane, Gouws & Kistner, 1969; Latham, Sutton & Sharpe, 1974). Division into species has proved difficult and only one species, *Butyrivibrio fibrisolvens*, is recognized (*Bergey's Manual of Determinative Bacteriology*, 1974). Shane *et al.* (1969) proposed that butyrivibrios be divided into two groups according to the relative proportions of their fermentation products. Group 1 strains utilize acetic acid and produce large amounts of lactic and small amounts of formic acids; Group 2 strains produce acetic acid and relatively small amounts of lactic and large amounts of formic acids. Most strains isolated belong to Group 1.

Experiments involving infusion of ¹⁴C-labelled volatile fatty acids into the rumen have demonstrated that considerable interconversion of acetate and n-butyrate carbon occurs *in vivo* (Leng, 1970) and recently Emmanuel, Milligan & Turner (1974) found extensive incorporation of ¹⁴C from [1-¹⁴C]acetate into rumen microbial protein, nucleic acid and lipids. It is possible that Group 1 butyrivibrios are involved in the metabolism of acetate within the rumen. The fermentation products of butyrivibrios are influenced by changes in pH and in the proportion of rumen fluid in the growth medium (Lee & Moore, 1959). We describe here cultural factors affecting acetate uptake or production in batch and continuous culture by strains of *B. fibrisolvens* which resemble Shane's Group 1 butyrivibrios.

METHODS

Organisms. The type strain of *B. fibrisolvens*, D₁, and strain 49 were provided by Dr M. P. Bryant, and strain K10 (K10-02-04-6B) was provided by Dr N. O. van Gylswyk. Strains NOR37 and IL631 were previously isolated in this laboratory (Sharpe & Reiter, 1972).

Media. The Hungate technique of anaerobic culture (Bryant & Robinson, 1961) was used throughout and all incubations were at 39 °C for 48 h unless stated otherwise. Cultures were maintained on medium 10 agar slants (Caldwell & Bryant, 1966). A medium containing rumen fluid and 0.5 % (w/v) cellobiose (Kistner, 1960; Shane *et al.*, 1969) was used to determine to which of Shane's two fermentation groups the strains belonged.

The basal synthetic (BS) medium used for all other growth studies contained (per 100 ml): 0.2 g trypticase (BBL); 0.4 g yeast extract (Difco); 7.5 ml mineral solution I; 7.5 ml mineral solution II; 0.1 ml of 0.1 % (w/v) resazurin solution. The compositions of the mineral solutions were: solution I, K₂HPO₄ (0.6 %, w/v); solution II, KH₂PO₄ (0.6 %, w/v); NaCl (1.2 %, w/v); (NH₄)₂SO₄ (1.2 %, w/v); MgSO₄·7H₂O (0.25 %, w/v); CaCl₂ anhydrous (0.12 %, w/v). Medium ingredients were made up to the appropriate volume with water and the pH was adjusted to 6.8 with 1.0 M-NaOH before autoclaving. Sterile 8.0 % (w/v) Na₂CO₃ (5.0 ml) and sterile 2.5 % (w/v) cysteine-HCl (2.0 ml) were added to the medium after autoclaving. None of the strains grew in the basal medium alone. Volatile fatty acids (VFA), acetic, propionic and n-butyric acids, were added individually, or as a mixture together with isobutyric, isovaleric and n-valeric acids, before adjustment of pH, to give final concentrations as used in medium 10, except where otherwise indicated. In the absence of added acetic acid, the medium contained 3 to 5 µmol acetate/ml from an impurity in the trypticase. Glucose, cellobiose and maltose were added as separate sterile solutions after autoclaving. The BS medium with added acetic acid (medium BSA) was used for the batch and continuous culture studies with glucose as the sole energy source. For these studies, the BSA medium was autoclaved and gassed with oxygen-free CO₂ on removal from the autoclave. Glucose, sodium carbonate and cysteine-HCl solutions were added and the medium allowed to equilibrate to 39 °C, with continuous gassing with oxygen-free CO₂, before inoculation. BSA medium with a low availability of CO₂ was prepared using 0.2 M-phosphate-citrate buffer, pH 6.8, reducing the final concentration of sodium carbonate to 0.04 % (w/v), and using 100 % oxygen-free N₂ as the headspace gas.

Apparatus. Batch-culture studies were made using 3 l Erlenmeyer flasks closed with rubber stoppers fitted with ports for gas inlet and outlet, medium sampling and inoculation. Continuous-culture studies were made using a New Brunswick C30 chemostat (New Brunswick Scientific Co., New Brunswick, New Jersey, U.S.A.) modified as follows: all silicone rubber tubing was replaced with butyl rubber tubing except for the medium delivery line where it passed around the pump; the medium reservoir, the headspace of the growth vessel and the spent medium collection vessel were gassed with independently controlled streams of oxygen-free CO₂; traps chilled in ice were incorporated in the effluent gas lines to remove moisture which would otherwise have condensed in the filters; gas filters were made from 150 × 25 mm glass tubing packed with glass wool. Sampling was achieved by temporarily diverting the outflow from the growth vessel to a side arm fitted with a tap.

Analysis. Optical densities of broth cultures were determined at 660 nm using a Cecil CE272 spectrophotometer (Cecil Instruments, Cambridge).

Acetic, propionic and n-butyric acids were determined by gas-liquid chromatography using a Perkin Elmer 881 chromatograph fitted with a flame ionization detector (Perkin Elmer, Beaconsfield, Buckinghamshire). Cell-free culture supernatant (1 ml) was acidified

with 0.6 M-orthophosphoric acid (0.05 ml) and n-pentanol [0.1 ml of a 0.25 % (v/v) solution] was added as an internal standard. The components, in 2 μ l subsamples, were separated on a 6 ft $\times$ $\frac{1}{8}$ in stainless-steel column packed with Chromosorb 80-100, polyethylene glycol 400 and orthophosphoric acid (18:2:0.1, by weight) at 115 °C with nitrogen (35 ml min⁻¹) as carrier gas.

Formic acid (Rabinowitz & Pricer, 1965), L(+)-lactic acid (Garvie, 1967), ethanol (Bonnichsen, 1965) and glucose (Sigma glucose oxidase kit) were determined enzymically. Total lactic acid was determined by the colorimetric method of Barker & Summerson (1941).

Hydrogen was determined from 1 ml of headspace gas by gas chromatography (Pye 104 chromatograph, katharometer detector; Pye Unicam, Cambridge) using a 5 ft $\times$ $\frac{1}{4}$ in glass column packed with silica gel (100 mesh) and using nitrogen (50 ml min⁻¹) as the carrier gas.

RESULTS

Grouping of strains using a rumen fluid medium

Strains NOR37, IL631 and 49 utilized acetic acid and produced large amounts of lactic acid and small amounts of formic acid when grown in the rumen fluid cellobiose medium (Table 1). On this basis they were classified as Group 1 organisms. Strain K10, previously characterized as a typical Group 2 organism (N. O. van Gylswyk, personal communication), was included for comparison. This strain produced acetic acid and more formic acid than the other three strains but considerably less lactic acid. The type strain, *B. fibrisolvens* D₁, could not be classified on the basis of its fermentation products in this medium since although lactic acid was a major product, acetate was produced and not utilized. Apart from strain IL631, all strains produced substantial amounts of hydrogen.

Acetate uptake or production using synthetic media

Level of yeast extract. In derivatives of the BS medium which did not contain any acetic acid other than that present in the trypticase, acetic acid was generally produced rather than utilized by the three Group 1 strains (Table 2). The relatively poor growth of strain 49 in this medium was enhanced by increasing the concentration of yeast extract to above 0.2 % (w/v), and the maximum optical density of all cultures was increased if 0.4 % (w/v) yeast extract was present. The factors in yeast extract which caused this growth stimulation have not yet been determined but of 12 vitamins tested, only biotin (40 μ g/10 ml medium) produced an increase in optical density when added to cultures grown in media containing 0.2 % (w/v) yeast extract. Whatever the level of yeast extract, the optical density of the cultures did not continue to increase once glucose had been depleted from the medium. Using high concentrations of yeast extract the amount of acetate produced by all strains was reduced.

Level of carbohydrate. When a VFA mixture was added to the medium containing a high concentration of yeast extract, the three Group 1 organisms NOR37, IL631 and 49 all utilized between 17.8 and 25.0 μ mol acetate/100 μ mol hexose. Acetate was also utilized when cellobiose or maltose were substrates (Table 2). In the presence of all three sugars the amount of acetate taken up was increased approximately in proportion to the additional carbohydrate fermented. In this case, the total amount of carbohydrate was not growth limiting. Strain D₁ utilized considerably less acetate than the other strains and produced small quantities of acetate when all three sugars were present in the medium.

When glucose was the only carbohydrate present, acetate utilization by the three Group 1 strains was greater if the amount of glucose in the medium was growth-limiting rather

Table 1. *Fermentation products of five strains of B. fibrisolvens grown in a rumen fluid cellobiose broth medium (Kistner, 1960)*

Product	Concentration of product ($\mu\text{mol}/100 \mu\text{mol hexose}$)				
	NOR37	IL631	49	D ₁	K10
Formic acid	33.7	26.7	27.3	49.0	62.3
Acetic acid	-20.0*	-39.1	-20.1	4.2	11.9
Propionic acid	0.7	0.0	0.4	0.0	0.4
n-Butyric acid	53.5	49.2	40.5	38.8	36.5
L(+)-Lactic acid	64.4	84.2	113.3	55.5	23.4
Ethanol	3.0	3.8	3.4	4.2	4.1
Hydrogen	57.0	2.3	37.1	32.3	38.1

* -, utilization.

Table 2. *Changes in acetate concentration after growth in batch culture of four strains of B. fibrisolvens in derivatives of BS medium*

Concentrations of glucose, cellobiose and maltose were such that, when added separately, they gave $25 \mu\text{mol hexose/ml}$ medium, and when added as a mixture, they gave $12.5 \mu\text{mol}$ each carbohydrate/ml medium.

Addition to BS medium					Concentration of acetate ($\mu\text{mol}/100 \mu\text{mol hexose}$)			
Glu	Cell	Mal	YE (%, w/v)	VFA (%, v/v)	NOR37	IL631	49	D ₁
+	0	0	0.1	0.0	7.9	12.1	9.3	7.2
+	0	0	0.4	0.0	0.0	7.2	6.8	3.0
+	0	0	0.4	0.31	-22.5*	-25.0	-25.0	-3.1
0	+	0	0.4	0.31	-22.5	-17.8	-22.8	-8.2
0	0	+	0.4	0.31	21.5	-21.4	-19.6	-7.2
+	+	+	0.4	0.31	-30.7	-29.3	-30.7	1.8

Glu, glucose; Cell, cellobiose; Mal, maltose; YE, yeast extract; VFA, volatile fatty acid solution.
0, not added to medium.

* -, utilization.

Table 3. *Changes in acetate concentration after growth in batch culture of four strains of B. fibrisolvens in BSA medium containing $10 \mu\text{mol}$ added acetate/ml and growth-limiting and non-limiting amounts of glucose*

	Concentration of glucose ($\mu\text{mol}/\text{ml}$ medium)	Concentration of acetate ($\mu\text{mol}/100 \mu\text{mol hexose}$)			
		NOR37	IL631	49	D ₁
Limiting	5.0	-16.8*	-17.0	-15.2	-6.0
Limiting	10.0	-13.1	-18.2	-19.2	3.0
Limiting	25.0	-15.7	-21.2	-19.9	-2.9
	Mean	-15.2	-18.8	-18.1	ND
Non-limiting	45.0	-6.8	-3.6	-5.2	2.2

* -, utilization.

ND, not determined.

than in excess (Table 3). In contrast to results with the mixed sugars, less acetate was utilized with a non-limiting glucose concentration ($45 \mu\text{mol ml}^{-1}$). Acetate uptake or production by strain D₁ was variable at all glucose concentrations.

Effect of VFA, sodium lactate and low initial pH. The addition of propionic or n-butyric acids to the BSA + glucose medium had little effect on acetate uptake. However, the addition

Table 4. *Changes in acetate concentration after growth in batch culture of four strains of B. fibrisolvens in BSA medium containing high or low levels of available CO₂ and in the presence or absence of sodium lactate*

The BSA medium contained added acetate and glucose at concentrations of 10 and 25 $\mu\text{mol/ml}$ medium respectively. High levels of available CO₂ were provided by using HCO₃⁻/CO₂ buffer, pH 6.8, and 100 % CO₂ as the headspace gas; low levels were provided by using 0.2 M-phosphate-citrate buffer, pH 6.8, 0.04 % (w/v) Na₂CO₃ and 100 % N₂ as the headspace gas.

Available CO ₂	Sodium lactate ($\mu\text{mol/ml}$)	Concentration of acetate ($\mu\text{mol}/100 \mu\text{mol hexose}$)			
		NOR37	IL631	49	D ₁
High	0	-27.5*	-17.9	2.5	3.2
High	30.0	-32.5	-19.6	-8.6	1.0
Low	0	-26.4	-21.8	-5.0	0.0
Low	30.0	-39.0	-25.7	-11.1	-12.9

* -, utilization.

of sodium lactate (30 $\mu\text{mol ml}^{-1}$) caused a small but consistent stimulation of acetate uptake by NOR37 and IL631 (Table 4). Strain 49 changed from being a weak acetate producer to being an acetate consumer. Reducing the availability of CO₂ also stimulated acetate utilization by strains IL631 and 49 and reduced the amount of acetate produced by D₁. When sodium lactate was incorporated into this low CO₂ medium, acetate uptake by all strains was markedly stimulated compared with the control BSA + glucose medium, but the growth rate of the organisms was reduced. None of the organisms was capable of fermenting lactic acid as a sole energy source but small quantities of lactate (< 3 $\mu\text{mol}/100 \mu\text{mol hexose}$) were apparently taken up from the medium when NOR37 was grown in the presence of glucose, acetic acid and sodium lactate.

The terminal pH of cultures grown in BSA + glucose medium was rarely less than 6.6 and never below 6.4. The effect of the initial pH of the medium on acetate utilization varied between strains. For example, with BSA medium containing glucose (25 $\mu\text{mol ml}^{-1}$) acetate consumption by NOR37 increased by 15 $\mu\text{mol}/100 \mu\text{mol hexose}$ when the initial pH of the medium was 6.2. None of the other strains grew well in media having an initial pH below 6.4, but, at this pH, strain IL631 utilized an additional 5.2 $\mu\text{mol acetate}/100 \mu\text{mol hexose}$. The growth rate and maximum optical density of all cultures were reduced by growth in media of pH 6.4 or below.

Release of fermentation acids during batch culture. In view of the variation in the extent of acetate uptake shown by individual strains, batch cultures in BSA medium containing 10 $\mu\text{mol acetate/ml}$ and 25 $\mu\text{mol glucose/ml}$ were monitored for changes in the concentration of acetic, n-butyric and lactic acids throughout the growth cycle. Using this medium, the release of fermentation acids during the growth of all strains followed the same general pattern, shown in Fig. 1(a) for strain NOR37. During the exponential phase of growth (exponential growth rate, 1.87 generations/h), n-butyric, lactic and acetic acids accumulated rapidly. On entering the stationary phase, when the glucose had been depleted almost to zero, acetate uptake commenced while n-butyrate synthesis continued, though at a much reduced rate. Surprisingly, negative changes in net acetate concentration were not always obtained during the incubation period. An increase in the concentration of acetate added to the medium to 15 $\mu\text{mol ml}^{-1}$ or above gave rise to a second pattern of fermentation with strain D₁ (Fig. 1b) in which acetate utilization started from the onset of growth and continued throughout exponential growth. The growth rate, 0.97 generations/h, was lower than in the

Fig. 1. Growth curves and acids produced or utilized during batch growth of *B. fibrisolvens* on BSA medium containing 25 μmol glucose/ml and (a) 10 μmol acetate/ml, strain NOR37, or (b) 15 μmol acetate/ml, strain D₁. ●, Acetic acid; ○, n-butyric acid; △, lactic acid. The broken line indicates the growth curve, determined by direct counts.

Table 5. Acetate uptake or production by five strains of *B. fibrisolvens* grown in batch culture in BSA medium containing 25 μmol glucose/ml and various amounts of added acetic acid

Concn of acetic acid ($\mu\text{mol}/\text{ml}$ medium)	Concentration of acetate ($\mu\text{mol}/100 \mu\text{mol}$ hexose)				
	NOR37	IL631	49	D ₁	K10
0	1.1	7.2	7.6	10.8	16.2
5	-14.4*	-16.6	0.7	10.8	20.9
10	-18.1	-18.0	-4.0	2.2	14.8
15	-26.4	-28.9	-20.6	-23.1	18.4
25	-30.3	-35.4	-19.5	-24.9	18.4
50	-37.2	-30.7	-21.7	-30.0	18.1

* —, utilization.

previous medium. Butyrate and lactate were produced and the rate of production of butyric acid was proportional to the rate of growth. On entering the stationary phase, the organism no longer utilized acetate and stopped producing the other acids.

Influence of concentration of extracellular acetate. Since increasing the concentration of acetate in the medium caused D₁ to shift from one type of fermentation to the other, the possibility that some threshold concentration of acetate was required in order to stimulate acetate uptake was investigated. The organisms, including for comparison the Group 2 strain K10, were grown for 48 h in BSA + glucose (25 $\mu\text{mol ml}^{-1}$) medium with 0 to 50 μmol acetic acid/ml. All strains produced acetate when grown in the absence of added acetate (Table 5) and strains NOR37 and IL631 utilized acetate from all the media containing added acetate. However, strains D₁ and 49 did not utilize acetate unless the initial concentration of acetic acid in the medium exceeded 10 $\mu\text{mol ml}^{-1}$ and 5 $\mu\text{mol ml}^{-1}$, respectively. The Group 1 organisms tended to utilize more acetate as its concentration was increased. The Group 2 organism, K10, produced acetate in all media whether acetate was added or not.

Continuous-culture studies. Results from batch-culture experiments suggested that the rate of growth of butyrivibrios might have a considerable influence on the extent of acetate uptake. This was examined using strain NOR37 grown in continuous culture in BSA + glucose

Fig. 2. Production or utilization of fermentation acids and ethanol by *B. fibrisolvens* strain NOR37 grown in continuous culture at different dilution rates. Medium was BSA + glucose ($27.8 \mu\text{mol ml}^{-1}$) containing acetic acid ($15 \mu\text{mol ml}^{-1}$). ●, Acetic acid; ○, n-butyric acid; ▲, formic acid; △, lactic acid; □, ethanol.

($27.8 \mu\text{mol ml}^{-1}$) medium containing $15 \mu\text{mol}$ added acetate/ml. At low dilution rates (D) of 0.05 to 0.1 h^{-1} , there was considerable acetate uptake (Fig. 2). Butyric and lactic acids were the major fermentation products, with formic acid and ethanol as minor components, but as the dilution rate was increased above 0.1 h^{-1} acetate uptake decreased and the amount of formate produced increased. The production of n-butyric and lactic acids and ethanol all decreased as the dilution rate increased with the result that, at a dilution rate of 0.4 h^{-1} , formic acid was the major fermentation product with butyric and lactic acids as secondary products. The organism entered a phase of relatively vigorous acetate production which reached $23 \mu\text{mol}/100 \mu\text{mol}$ hexose as the dilution rate increased above 0.235 h^{-1} . The mean carbon recovery was 105 % for all samples except those taken at the lowest dilution rate where an exceptionally high, and so far unexplained, value (122 %) was obtained. Production of hydrogen was not measured.

DISCUSSION

When grown in a medium containing rumen fluid, three of the four strains examined, NOR37, IL631 and 49 could be classified as Group 1 butyrivibrios on the basis of acetate uptake, low formate and high lactate production (Shane *et al.*, 1969). Compared with the initial characterization of this strain (Bryant & Small, 1956), strain D_1 behaved atypically in not utilizing acetate from the rumen fluid medium. Many interrelated factors influence the uptake of acetate. Earlier work by Lee & Moore (1959) indicated that in the presence of rumen fluid the uptake was related to the amount of rumen fluid in the medium and to the initial pH of the medium. The results presented here suggest that four other factors may influence acetate uptake: the level of carbohydrate; the presence of sodium lactate; the availability of CO_2 ; and the rate of growth of the organism. For some strains, such as D_1 , a

more important factor may be the concentration of extracellular acetate. Of these factors, low pH, the presence of sodium lactate, low availability of CO_2 and a high concentration of acetate (or rumen fluid) may be the factors which stimulate acetate uptake by reducing the growth rate of the organism.

This relationship between net acetate uptake and growth rate was demonstrated when strain NOR37 was grown in continuous culture under energy (glucose) limitation in a medium of constant pH and high availability of CO_2 . As the dilution rate, and hence growth rate, increased, so the amount of acetate taken up decreased, with a corresponding fall in butyrate production. Assuming that, as suggested by Joyner & Baldwin (1966), these strains possess a coli-type phosphoroclastic system, the production of formate at all dilution rates indicates that the cleavage of pyruvate to acetate and formate proceeds even during phases of acetate uptake. The observed production or utilization of acetate may therefore be the net result of the two activities. The increasing amounts of formate synthesized at increasing growth rates suggest that the phosphoroclastic cleavage of pyruvate becomes more rapid under these conditions. If this is so, then the behaviour of an organism as a Group 1 or Group 2 butyrivibrio may depend in part upon the rate of this reaction.

Variations in acetate uptake or formation by strain D_1 were particularly marked. Much of the variability encountered with this strain in early experiments may have been due to the initial concentration of acetate in the medium being at, or around, a threshold value (10 to 15 μmol acetate/ml medium), below which acetate was a product of glucose fermentation, and above which acetate was utilized. The maximum total concentration of acetate reached in batch fermentation as the organism approached the stationary phase corresponded to this threshold value (Fig. 1a).

On the basis of the batch- and continuous-culture studies with NOR37, butyrate production and acetate utilization appeared to be linked. In batch culture, when determining the acetate threshold concentration for strain D_1 , the change from acetate production to utilization and the increase in butyrate production were 50 and 24 $\mu\text{mol}/100 \mu\text{mol}$ respectively, i.e. in the ratio 2:1. Similarly, using values taken from the continuous culture data, at dilution rates of 0.05 h^{-1} (maximum acetate uptake) and 0.235 h^{-1} (no net acetate uptake or production), the changes in acetate uptake and butyrate production were 36 and 18 $\mu\text{mol}/100 \mu\text{mol}$ hexose, respectively. Since the normal route of butyrate synthesis involves the formation of acetoacetyl CoA from two moles of acetyl CoA, it is tempting to assume that a significant proportion of the butyrate carbon synthesized during the phase of acetate uptake is derived from extracellular acetate. If extracellular acetate is used for electron disposal in this way, then to obtain any energetic advantage the organism must link the two units of reoxidized cofactors so formed to glycolysis to allow another mole of hexose to be oxidized. In those cultures in which acetate-butyrate conversions appeared to continue in the stationary phase, the necessary reducing equivalents may have arisen from the turnover of some cellular storage compound formed when glucose was still in excess. The exact fate of acetate carbon can only be determined by tracer studies. From the work of Kistner & Kotzé (1973) it seems probable that Group 1 butyrivibrios are able to carry out reductive carboxylation reactions and contain a pyruvate synthetase. Randomization of some acetate carbon into lactic acid or amino acids might therefore be expected to occur.

Butyrivibrio fibrisolvens is numerically one of the most important organisms in the rumen of the roughage-fed animal. The dilution rate of the rumen fluid of such an animal is unlikely to exceed 0.1 h^{-1} and the concentration of acetate in the fluid will be at least four or five times higher than the 15 $\mu\text{mol ml}^{-1}$ *in vitro* threshold level found for strain D_1 . Satter & Esdale (1968) demonstrated that randomization of acetate carbon into butyrate was much

greater in rumen fluid taken from animals fed with roughage than in fluid from animals fed with concentrates. It is probable that under conditions of high roughage feeding, *B. fibrisolvens* may be among the most active organisms contributing to turnover of VFA within the rumen.

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