Appl Microbiol Biotechnol (1992) 36:592-597

Applied and Microbiology Biotechnology

Glycerol conversion to 1,3-propanediol by newly isolated clostridia

Hanno Biebl[1], Sabine Marten[1], Hans Hippe[2], and Wolf-Dieter Deckwer[1]

[1] Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, W-3300 Braunschweig, Federal Republic of Germany
[2] Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, W-3300 Braunschweig, Federal Republic of Germany

Received 5 September 1991/Accepted 31 October 1991

Summary. From pasteurized mud and soil samples glycerol-fermenting clostridia that produced 1,3-propanediol, butyrate and acetate were obtained. The isolates were taxonomically characterized and identified as *Clostridium butyricum*. The most active strain, SH1 = DSM 5431, was able to convert up to 110 g/l of glycerol to 56 g/l of 1,3-propanediol in 29 h. A few *Clostridium* strains from culture collections (3 out of 16 of the *C. butyricum* group) and some isolates of Kutzner from cheese samples were also able to ferment glycerol, but the final concentration and the productivity of 1,3-propanediol was lower than in strain SH1. Strain SH1 grew well in a pH range between 6.0 and 7.5, with a weak optimum at 6.5, and was stimulated by sparging with N_2. Best overall productivity was obtained in fed-batch culture with a starting concentration of 5% glycerol. In all fermentations the yield of 1,3-propanediol in relation to glycerol was higher than expected from NADH production by acid formation. On the other hand the H_2 production was lower than expected, if per mole of acetyl coenzyme A one mole of H_2 is released. The observations point to a substantial transfer of reducing potential from ferredoxin to NAD, which finally results in increased 1,3-propanediol production.

Introduction

More and more detergents are presently produced in the developing countries from natural fats. Following saponification or transesterification the fatty acids are chemically modified for the desired product while the glycerol is left over. As a consequence the prices for glycerol have fallen drastically. In this situation a biological process could be helpful by which glycerol is converted to another chemical in more demand, particularly if the process water from fat transesterification can be used without prior purification.

Offprint requests to: H. Biebl

It has been known for about 50 years, but rarely mentioned in textbooks, that glycerol is fermented by facultatively anaerobic bacteria to 1,3-propanediol, ethanol, 2,3-butanediol, acetic and lactic acids. Of these substances 1,3-propanediol is of interest as a monomer for light-insensitive plastics, and some strains indeed form this glycol as the main product. Suitable organisms are known to occur among the enterobacterial genera *Klebsiella* and *Citrobacter* (Homann et al. 1990). In recent years it has been shown that some clostridial species also convert glycerol to 1,3-propanediol in an appreciable yield. The fermentation pattern is different in that the clostridia form butyric acid as a by-product (Forsberg 1987; Heyndrickx et al. 1991). Some strains of *C. pasteurianum* form higher amounts of butanol and ethanol in addition (Nakas et al. 1983; Heyndrickx et al. 1991).

In this paper we try to evaluate the clostridial glycerol fermentation with respect to the attainable concentration and yield of 1,3-propanediol. From specific enrichment cultures for spore-forming anaerobic glycerol utilizers, actively fermenting strains were selected, compared to the corresponding existing strains and optimized for culture conditions. In comparison to enterobacterial glycerol fermentation, the 1,3-propanediol yield sould be diminished in the clostridial fermentation by the production of butyric acid, which provides less reducing potential than acetate. On the other hand, reducing potential from pyruvate decarboxylation could be made available for propanediol formation rather than being released as molecular H_2. This problem is treated in a section on the stoichiometry of the process.

Materials and methods

Organisms. C. butyricum AK1, SH1, OS1 and OS3 were obtained as isolates from compost (AK), decaying straw (SH) and river mud (OS). AK1 and SH1 were deposited with the German Collection of Microorganisms (DSM) as DSM 5430 and DSM 5431 as patent strains (Kretschmann et al. 1989). Strain NRRL B593 was

Table 1. Fermentation of 2% glycerol by isolated and known *Clostridium* strains

Strains		Fermentation time (h)	Products (mol/100 mol glycerol)			
			1,3-Propanediol	Acetic acid	Butyric acid	Ethanol
C. butyricum	SH1 (DSM 5431)	9.0	62	20	7	0
	AK1 (DSM 5430)	9.5	62	11	4	0
C. butyricum	W3α	9.5	71	15	7	0
C. "multifermentans"	Lille	10.5	70	15	7	0
C. "kainantoi"	DSM 523	14.0	59	5	11	1
C. butyricum	DSM 2478	19.0	58	3	9	2

kindly provided by C. W. Forsberg, Guelph, Canada; NRC 33007 was purchased from the National Research Council, Ottawa, Canada. Both strains were designated as *C. "butylicum"*. All other strains were obtained from the DSM.

Medium and culture conditions. These were described previously (Biebl 1991; Homann et al. 1990). Batch cultures for process optimization were performed in 1-l fermentors (BCC, Göttingen, FRG) under pH control. The culture pH was 7.0 and the growth temperature 32° C. Fed-batch cultures were started with 5% glycerol; before complete consumption, glycerol was repeatedly added from an autoclaved 87% solution to give a concentration of 2% in the culture.

Analytical methods. Glycerol was determined enzymatically using the test kit and instructions of Boehringer (Mannheim, FRG). Fermentation products were determined gas chromatographically with Chromosorb 101 as the column material (Biebl 1991). H_2 in the exit gas was measured using a gas chromatograph (Chrompack 437 A) with a thermal conductivity detector and a 2-m stainless steel column filled with molecular sieve (0.5 nm). Calibration was done with gas mixtures ($N_2/CO_2/H_2$) purchased from Messer Griesheim (FRG). CO_2 was followed by means of an infrared CO_2 analyser (Unor 4N, Maihak, Hamburg, FRG).

Results

Enrichment and isolation

Several pasteurized soil and mud samples were inoculated into O_2-free 2% glycerol medium. After 2–3 days' incubation at 30° C, in most of the bottles dense cultures of large, sometimes spore-forming cells had developed. They had produced 1,3-propanediol in concentrations of 8–15 g/l at a final pH of 4.8–5.0. Generally speaking the samples from decomposing plant material and from river mud gave the most active cultures, whereas enrichments from garden and wood soils developed poorly or failed.

After repeated application of the agar shake culture technique (Pfennig 1978), pure cultures were obtained of which four were selected for further characterization. The cells of the four strains were almost alike: they measured 0.9–1.5 × 3.2 μ and formed oval subterminal spores.

Identification

Using the test system of Holdeman and Moore (1977) and Cato et al. (1986) the four isolates could be attributed to *C. butyricum*. They proved to be identical in almost all characters with the main exception of starch and glycogen fermentation, which was positive for strains AK1 (DSM 5430) and OS1 and negative for SH1 (DSM 5431) and OS3, thus deviating from *C. butyricum*. All four strains were unable to produce acid from raffinose, ribose and melibiose, which would point to *C. acetobutylicum*. From this species, however, the strains are distinguished by their failure to liquefy gelatine, their ability to grow in a yeast-extract-peptone medium without carbohydrates and to grow with biotin as the only growth factor. *C. beijerinckii* was excluded, as the strains were able to grow in a mineral medium without complex substrates. Least agreement was found with *C. pasteurianum* because of an essentially different pattern of carbon sources.

The classification with *C. butyricum* was corroborated by analysis of the cellular fatty acids. The composition was very close to the type strain of *C. butyricum* (and of *C. beijerinckii*) and distant to that of *C. acetobutylicum*.

Glycerol fermentation by existing Clostridium strains

According to Holdeman and Moore (1977), glycerol utilization is scarcely distributed among the *Clostridium* species. Of 16 collection strains belonging to the *C. butyricum* group, only three were able to grow on glycerol, i.e. *C. butyricum* DSM 2477 and DSM 2478, two recent isolates utilizing pectin (Schink et al. 1981; Schink and Zeikus 1982), and *"C. kainantoi"* DSM 523, an acetone-butanol producing strain. The strains that did not grow on glycerol included *C. butyricum* DSM 522, *C. beijerinckii* DSM 53, DSM 791, and DSM 1820, *C. "butylicum"* NRRL B 593 and NRC 33007, *C. acetobutylicum* DSM 792, DSM 1731, DSM 1732, DSM 1737, DSM 1738 and DSM 1739 and *C. "saccharoperbutylacetonicum"* DSM 2152.

In addition, several *Clostridium* strains were available that had been unofficially deposited with the DSM

Table 2. Maximum glycerol utilization in fed-batch culture

Strains		Maximum glycerol concentration utilized (g/l)	1,3-Propanediol (g/l)	Acetic acid (g/l)	Butyric acid (g/l)	Fermentation time (h)
C. butyricum	AK1	98.3	56.4	1.7	9.9	34
	SH1	82.4	41.8	2.5	2.5	23
C. butyricum	W3α	57.5	20.4	2.8	2.8	40
C. "multifermentans"	Lille	51.1	20.6	0.9	0.9	34

and were listed as glycerol-utilizers (cf. Kutzner 1963). Two of them (*C. butyricum* W3α and *C. "multifermentans"* Lille) showed good growth on glycerol, three (*C. "saccharobutyricum"* SB 55, S 10 and Lille) grew moderately, and one did not grow (*C. butyricum* W 100). All positive strains formed 1,3-propanediol, acetate, and butyrate in a similar proportion as with the isolates.

Productivity and concentration of 1,3-propanediol obtained in isolated and existing strains

Four of the DSM strains that showed best growth in bottle cultures were compared with two of the isolates in 0.7-l batch cultures at a controlled pH of 7.0 (Table 1). The isolates AK1 (DSM 5430) and SH1 (DSM 5431) and the two Kutzner strains converted 2% glycerol in about 10 h, whereas the two catalogue strains were markedly slower. No significant differences were found in product composition except for the acetic acid/butyric acid proportion.

The four strains that grew equally well with 2% glycerol were grown in fed-batch culture to ascertain the maximum concentration of glycerol that could be converted. It turned out that the isolates were by far superior to the existing strains (Table 2). Although strain SH1 fermented glycerol and produced 1,3-propanediol in lower concentration than AK1 in these experiments, it was used as the representative strain for further optimization because of its faster growth.

Table 3. Productivity of 1,3-propanediol at increasing initial glycerol concentration by *C. butyricum* SH1 (DSM 5431)

Culture	Glycerol fermented (g/l)	1,3-Propanediol produced (g/l)	Fermentation time (h)	Overall productivity (g/l/h)
Batch	26	15.0	9.5	1.6
Batch	52	29.5	13.0	2.3
Batch	110	56.0	29.0	1.9
Fed-batch	82	47.0	21.0	2.2

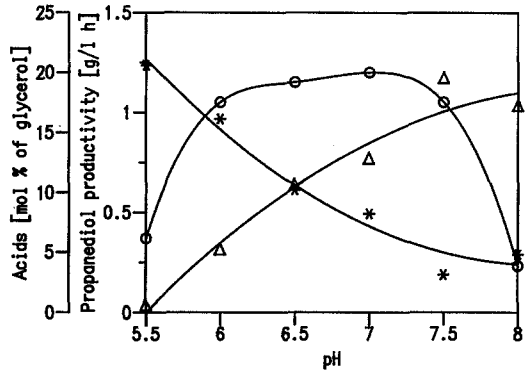

Fig. 1. Overall productivity of 1,3-propanediol (○) and the content of acetic (△) and butyric (*) acids in batch cultures of *Clostridium butyricum* DSM 5431 grown at various pH values

Table 4. Influence of agitation and nitrogen sparging on the fermentation of 2% glycerol by *C. butyricum* SH1

Agitation rate (rpm)	Sparging with N_2 (0.08 vvm)	Fermentation time (h)	KOH consumed (mol/100 mol of glycerol)	Products (molar percentage of glycerol consumed for their formation)			
				1,3-Propanediol	Butyric acid	Acetic acid	Sum (recovery)
100	–	11	44	69	13	14	97
400	–	11	44	62	12	13	90
100	–	10	45	65	10	16	92
100	+	9.5	28	64	11	16	92
100	–	9.5	41	66	10	16	92
400	+	7.5	33	62	15	14	91

Each fermentor pair was inoculated from the same preculture. The pH was controlled at 7.0 in all cultures

Optimum fermentation conditions for C. butyricum SH 1 (DSM 5431)

The influence of the initial glycerol concentration on the fermentation time and overall productivity of 1,3-propanediol is shown in Table 3: 5% glycerol was more effectively converted than 2.5%, but at an initial concentration of 11% productivity decreased because of an extended lag phase. This lag phase could be avoided by successive addition of glycerol, so that the productivity of the 5% glycerol culture could be maintained. The lower final propanediol content in the fed-batch culture shown in Table 3 does not seem to be significant. In later experiments with an elaborated feeding strategy propanediol concentrations of 55 g/l were regularly obtained (Günzel et al. 1991).

As shown in Fig. 1 good production of 1,3-propanediol proceeded in the relatively wide pH range between 6.0 and 7.5. Interestingly, acid formation shifted from butyric acid at low pH to acetic acid at high pH. It should be mentioned, however, that fermentations deviating from this finding occurred, particularly in fermentations running slower than usual.

The fermentor cultures treated up to this point were slowly agitated (100 rpm) and not sparged with inert gas except for the first 1 or 2 h after inoculation. It was shown in three pH-controlled batch cultures, each paralleled by an equally inoculated reference culture, that fermentation was accelerated to some extent when the culture was sparged with N_2, but not by increased stirrer speed alone (Table 4). Decreased alkali consumption in N_2-sparged cultures indicates that the effect is due to expulsion of CO_2. The acid content and proportion remained almost uninfluenced.

Stoichiometry

Conversion of glycerol to the respective products can be written as:

$$\text{glycerol} \rightarrow \text{acetate} + CO_2 + H_2 + 4\,[H] \quad (1a)$$

$$2\ \text{glycerol} \rightarrow \text{butyrate} + 2\,CO_2 + 2\,H_2 + 4\,[H] \quad (1b)$$

$$\text{glycerol} + 2\,[H] \rightarrow \text{1,3-propanediol} \quad (1c)$$

where [H] indicates reducing equivalent. In batch fermentations growing well and in substrate-sufficient continuous cultures (Biebl 1991) the molar proportion of acetic acid and butyric acid was found to be 2:1. The fermentation equation is then:

$$10\ \text{glycerol} \rightarrow 2\ \text{acetate} + \text{butyrate} + 6\ \text{1,3-propanediol} + 4\,CO_2 + 4\,H_2 \quad (2)$$

Thus, in cultures without cell production 1,3-propanediol should be obtained with a yield of 60% (mol/mol). In growing cultures the yield decreases by additional glycerol requirement for cell mass, but increases by additional 1,3-propanediol formation as a result of NADH formation coupled to cell mass formation. Using a cell mass formula of $C_4H_7O_2N$ ($CH_{1.86}O_{0.43}N_{0.24}$ was actually determined) the conversion of glycerol to cell mass can be formulated as:

$$\tfrac{8}{3}\ \text{glycerol} + NH_3 \rightarrow (C_4H_7O_2N) + \tfrac{4}{3}\ \text{1,3-propanediol} \quad (3)$$

The amount of cell mass formed during fermentation of 10 mol glycerol (Eq. 2) can be calculated from the Y_{ATP} value, which was determined by Günzel (1991) as 8.5 g cell mass/mol ATP. As seven moles of ATP are formed in Eq. 2 (four moles for each mole of glycerol transformed to acids and three moles with acid formation) the cell mass produced is 59.5 g or 59.5:101 = 0.59 "mol" (101 = relative molecular mass). According to Eq. 3, 1.57 mol glycerol are required for this cell mass, and 0.79 mol 1,3-propanediol are released. Combining

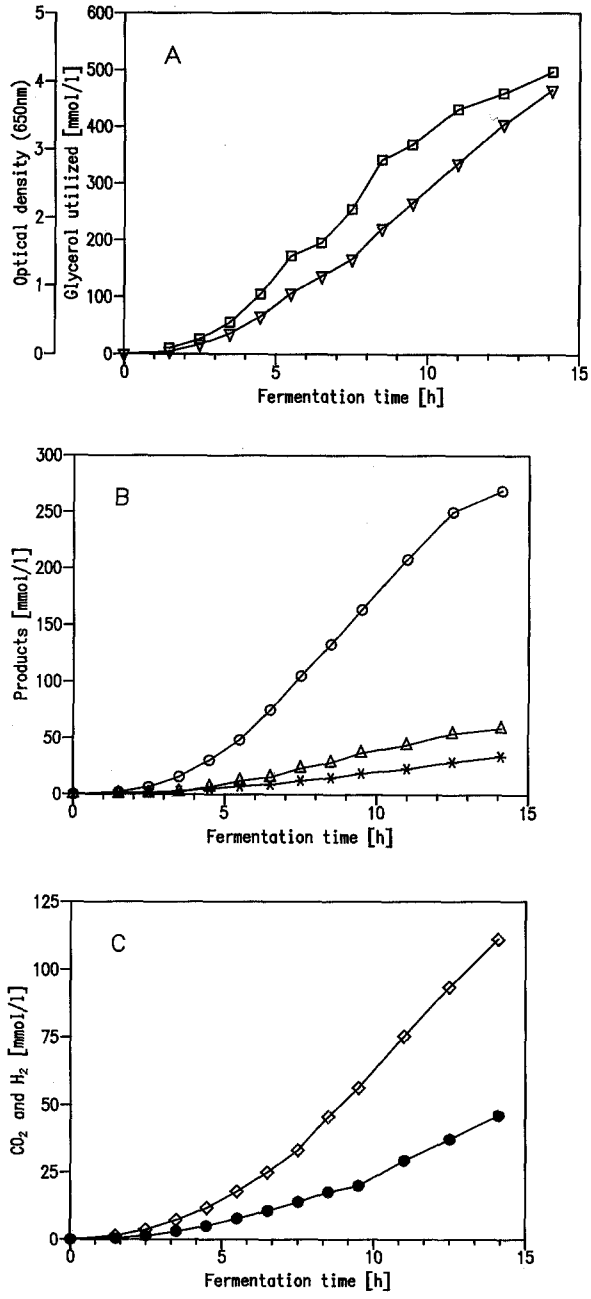

Fig. 2. Growth, substrate utilization (**A**) and formation of liquid (**B**) and gaseous products (**C**) in a batch culture of *C. butyricum* DSM 5431. **A** Optical density (□); glycerol utilized (▽). **B** 1,3-Propanediol (○); acetic acid (△); butyric acid (*). **C** CO_2 (◇); H_2 (●)

Table 5. Glycerol consumption and product formation in a batch culture with measurement of gas production after 12.5 h

Parameter	Amount per litre	Molar percentage
Glycerol consumed	403.8 mmol	
Cell mass	1.3 g	
1,3-Propanediol	250.0 mmol	62.0 of glycerol consumed
Acetic acid	54.2 mmol	13.4 of glycerol consumed
Butyric acid	29.2 mmol	14.5 of glycerol consumed
Ethanol	1.8 mmol	0.4 of glycerol consumed
CO_2	93.4 mmol	82.9 of calculated CO_2
H_2	37.2 mmol	33.0 of calculated H_2
Glycerol for products	364.4 mmol	90.2 of glycerol consumed
Glycerol for cell mass	17.1 mmol	4.2 of glycerol consumed
1,3-Propanediol calculated[a]	259.3 mmol	103.7 of propanediol measured

[a] From acids, H_2, and cell mass production

Eqs. 2 and 3, the final relation between glycerol, cell mass and 1,3-propanediol is:

$$11.57 \text{ glycerol} \rightarrow 0.59 \ (C_4H_7O_2N) + 6.79 \text{ 1,3-propanediol} \quad (4)$$

the propanediol yield being 58.7%.

In the majority of fermentations the theoretical yield was exceeded by about 2–7%. It was therefore suspected that part of the H_2 assumed to be released as molecular H_2 serves to reduce additional glycerol to 1,3-propanediol. This was checked in a batch culture with a complete record of all products including the gases. A 2.5-l fermentor culture with 5% glycerol was set up with continuous measurement of H_2 and CO_2 in an N_2 gas stream. Figure 2 shows the kinetics of this fermentation and Table 5 the glycerol consumption and product formation after 12.5 h. It is clearly seen that H_2 was formed in a much lower amount than postulated in Eq. 2: only 33% of this amount was released if based on the acids or 40% if based on measured CO_2. If the reducing equivalents from acids, biomass, and acetyl CoA formation are summed up and subtracted by the molecular H_2 generated, a molar amount is found that is very close to the propanediol measured. With a release of one third of the possible H_2 amount, the reaction equation of a typical clostridial propanediol fermentation can now be written as:

$$14.24 \text{ glycerol} + 1 \ NH_3 \rightarrow 2 \text{ acetate} + 1 \text{ butyrate} + 9.46 \text{ 1,3-propanediol} + 4 \ CO_2 + 1.33 \ H_2 + 59.5 \text{ g cell mass} \quad (5)$$

resulting in a propanediol yield of 66.4%. The actually measured yield of 62.0% is somewhat lower, but higher than in case of maximum H_2 release (Eq. 4).

Discussion

Earlier (Homann et al. 1990) we found that anaerobic enrichment cultures from mud and soil samples with glycerol as the carbon and energy source led to either *Citrobacter* producing 1,3-propanediol and acetate or *Klebsiella* producing 1,3-propanediol, acetate, and ethanol. It has now been demonstrated that enrichment cultures from pasteurized samples with glycerol invariably yielded clostridia of the *Clostridium butyricum* type forming 1,3-propanediol, butyric and acetic acids as fermentation products. It appears that these fermentation types are widely distributed in nature and that the clostridia are usually suppressed in the presence of enterobacteria. A further glycerol fermentation type described in the literature (Nakas et al. 1983; Heyndrickx et al. 1991) in which butanol is the main product could not be obtained by enrichments (unpublished results).

Some *Clostridium* strains from the German Collection of Microorganisms were able to ferment glycerol to the same products as the isolates, two of *C. butyricum* and one probably related to *C. acetobutylicum (C. "kainantoi")*. Apart from strains from the collection of H. J. Kutzner for which glycerol utilization was already known, all other strains tested did not grow on glycerol including the type strain of *C. butyricum*. This is in contrast to the results of Forsberg (1987) who found 17 out of 21 strains selected among the *C. butyricum* group to be positive for growth on glycerol. Strangely, four of Forsberg's positive strains (DSM 53 = ATCC 14949, DSM 792 = ATCC 824, NRRL B 593, NRC 33007) did not grow in our hands, not even in the bicarbonate buffered medium used by the author. Comparable to our screening experiment was that of Heyndrickx et al. (1991), who found only one out of nine strains of *C. butyricum* to ferment glycerol unambiguously.

By our estimation glycerol fermentation among clostridia is not a property of a particular species but occurs sporadically in *C. butyricum* and related species. This is in accordance with the substrate utilization tables of Holdeman and Moore (1977), which indicate acid production from glycerol only for *C. butyricum* and for single strains of other species.

Table 6. Optimum fermentation parameters in clostridia (*C. butyricum* DSM 5431) and enterobacteria (*Klebsiella pneumoniae* DSM 2026)

Parameter	*Clostridium*	*Klebsiella*
Glycerol fermented (g/l)	110	125
1,3-Propanediol produced (g/l)	56	56
1,3-Propanediol yield (weight % of glycerol)	51	45
1,3-Propanediol productivity (g/l/h)	2.2	2.3

Glycerol fermentation to 1,3-propanediol has been physiologically investigated in enterobacteria of the genera *Klebsiella* and *Citrobacter* (see Forage and Foster 1982). It has been recently shown that the enterobacterial fermentation, known since 1940 (Mickelson and Werkman 1940), can be run very efficiently after appropriate strain selection and optimization of the culture conditions (Tag 1991; Homann et al. 1990). Table 6 compiles characteristical data of *K. pneumoniae* DSM 2026, the presently best enterobacterial strain, and of *Clostridium butyricum* DSM 5431. It is shown that both processes are comparable concerning achievable 1,3-propanediol concentration and productivity. However, *Klebsiella* forms more by-products that do not contribute to propanediol formation (ethanol) or only to a minor extent (lactate, 2,3-butanediol) and consequently require more glycerol. The composition of by-products may vary considerably in *Klebsiella* cultures, whereas in clostridia only the acetate/butyrate proportion is changed.

In all batch fermentations performed the 1,3-propanediol content was higher than expected from the reaction equation. When gas production was measured H_2 formation was lower than expected. This is consistent with the concept that a greater part of ferredoxin-bound H_2 is made available for further reduction of glycerol rather than being released as molecular H_2 via hydrogenase. The enzyme that transfers H_2 from ferredoxin to NAD has been demonstrated for the acetone-butanol fermentation (Petitdemange et al. 1976). In the batch culture described one third of the possible amount of H_2 was produced, while in a chemostat culture with *C. butyricum* IFO 3315 t_2, no H_2 was detected at all (Heyndrickx et al. 1991). Complete or partial utilization of ferredoxin-bound hydrogen for propanediol formation is another advantage over glycerol fermentation with enterobacteria that are unable to transfer reducing equivalents from pyruvate decarboxylation to NAD. Therefore, from the biotechnological viewpoint, 1,3-propanediol production from glycerol by clostridia appears to deserve further development.

References

Biebl H (1991) Glycerol fermentation to 1,3-propanediol by *Clostridium butyricum*. Measurement of product inhibition by use of a pH-auxostat. Appl Microbiol Biotechnol 35:701–705

Cato EP, George WL, Finegold SM (1986) Genus *Clostridium*. In: Sneath PHA, Mair NS, Scharpe ME, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 2. Williams and Wilkins, Baltimore, pp 1141–1200

Forage RG, Foster MA (1982) Glycerol fermentation in *Klebsiella pneumoniae*. Functions of the coenzyme B_{12}-dependent glycerol and diol dehydratases. J Bacteriol 149:413–419

Forsberg CW (1987) Production of 1,3-propanediol from glycerol by *Clostridium acetobutylicum* and other *Clostridium* species. Appl Environ Microbiol 53:639–643

Günzel B (1991) Mikrobielle Herstellung von 1,3-Propanediol durch *Clostridium butyricum* und adsorptive Abtrennung von Diolen. Ph. D. thesis, University of Braunschweig, FRG

Günzel B, Yonsel Ş, Deckwer W-D (1991) Fermentative production of 1,3-propanediol from glycerol by *Clostridium butyricum* up to a scale of 2 m^3. Appl Microbiol Biotechnol 36:289–294

Heyndrickx M, Vos P de, Vancanneyt M, Ley J de (1991) The fermentation of glycerol by *Clostridium butyricum* LMG 1212 t_2 and 1213 t_1 and *C. pasteurianum* LMG 3285. Appl Microbiol Biotechnol 34:637–642

Holdeman LV, Moore WEC (1977) Anaerobe laboratory manual. Virginia Polytechnic Institute, Blacksburg, Va.

Homann T, Tag C, Biebl H, Deckwer W-D, Schink B (1990) Fermentation of glycerol to 1,3-propanediol by *Klebsiella* and *Citrobacter* strains. Appl Microbiol Biotechnol 33:121–126

Kretschmann J, Caduck F-J, Deckwer W-D, Tag C, Biebl H (1989) Fermentative Herstellung von 1,3-Propanediol. German patent no. DE 3924423 A1

Kutzner HJ (1963) Untersuchungen an Clostridien mit besonderer Berücksichtigung der für die Milchwirtschaft wichtigen Arten. Zentralbl Bakteriol Parasitenk Infektionskr Hyg Abt 1 Orig 191:441–450

Mickelson MN, Werkman CH (1940) Formation of trimethylene glycol from glycerol by *Aerobacter*. Enzymologia 8:252–256

Nakas JP, Schaedle M, Parkinson CM, Coonley CE, Tanenbaum SW (1983) System development for linked-fermentation production of solvents from algal biomass. Appl Environ Microbiol 46:1017–1023

Petitdemange H, Cherrier C, Raval G, Gay R (1976) Regulation of NADH and NADPH-ferredoxin oxidoreductases in clostridia of the butyric group. Biochim Biophys Acta 421:334–347

Pfennig N (1978) *Rhodocyclus purpureus* gen. nov., sp. nov., a ring-shaped, vitamin B_{12}-requiring member the family Rhodospirillaceae. Int J Syst Bacteriol 28:283–288

Schink B, Zeikus JG (1982) Microbial ecology of pectin decomposition in anoxic lake sediments. J Gen Microbiol 128:393–404

Schink B, Ward JC, Zeikus JG (1981) Microbiology of wet wood: importance of pectin degradation and *Clostridium* species in living trees. Appl Environ Microbiol 42:526–532

Tag CG (1991) Mikrobielle Herstellung von 1,3-Propandiol. Ph. D. thesis, University of Oldenburg, FRG

Takeda Y, Matsui T (1955) Mycological studies of acetone-butanol fermenting bacteria (KN-18) (*Clostridium kainantoi* n. sp.). Agric Biol Chem 29:78–82