Appl Microbiol Biotechnol (1991) 36:289-294

Applied and Microbiology Biotechnology

Fermentative production of 1,3-propanediol from glycerol by *Clostridium butyricum* up to a scale of 2 m^3

Bernd Günzel, Sems Yonsel, and Wolf-Dieter Deckwer

GBF, Biochemical Engineering Division, Mascheroder Weg 1, W-3300 Braunschweig, Federal Republic of Germany

Received 3 June 1991/Accepted 12 August 1991

Summary. The conversion of glycerol to 1,3-propanediol (PD) by *Clostridium butyricum* DSM 5431 was studied in anaerobic culture. Growth and product formation were optimal at pH = 7.0 and $T = 35°C$, while aeration rate and stirrer speed were found to have no significant influence. As increasing amounts of initial glycerol led to inhibition of growth, cultivations were done in fed-batch operation. Comparative cultivations were carried out in an air-lift (ALR) and a stirred-tank reactor (STR) having equal working volumes ($V_L = 30$ l) and no difference in product formation was found. The process was scaled up to reactor sizes of 1.2 m^3 (ALR) and 2.0 m^3 (STR). The same results were obtained irrespective of reactor volume as well as reactor type (STR/ALR). PD concentrations of approximately 50–58 $g \cdot l^{-1}$ and overall productivities of 2.3–2.9 $g \cdot l^{-1} \cdot h^{-1}$ could be reached.

Introduction

1,3-Propanediol (PD) is a versatile intermediate compound for the synthesis of heterocycles and as a monomer for the production of polymers such as polyesters and polyurethanes. In addition, PD can advantageously be used as a solvent and additive for lubricants. The chemical conversion of acrolein into 1,3-propanediol is rather difficult and proceeds with low selectivity. Hence, PD is produced only in negligible amounts and its high price has prevented utilization of PD in polymer industries. On the other hand, the microbial conversion of glycerol to PD is relatively simple. In addition, glycerol is relatively cheap and its conversion to PD could help to reduce glycerol surpluses on the market.

PD is produced from glycerol by Enterobacteriaceae such as *Klebsiella* (Streekstra et al. 1987; Tran Dinh and Hill 1989; Homann et al. 1990) and *Citrobacter* (Gottschalk and Averhoff 1990; Homann et al. 1990). Bacteria such as *Lactobacillus* (Schütz and Radler 1984) and *Clostridium* (Nakas et al. 1983; Forsberg 1987) also convert glycerol to PD. The pathways to PD in *Enterobacter* have been described by Ruch and Lin (1975) and Forage and Foster (1982). The microbial conversion of glycerol to PD is a disproportionation. On the one hand, glycerol is dehydrated to 3-hydroxypropionaldehyde followed by hydrogenation to PD by $NADH_2$. On the other hand, glycerol is converted to pyruvate, which in turn reacts to various by-products (acetate, lactate, butyrate, ethanol, H_2, CO_2) with generation of $NADH_2$. The highest PD yield is obtained if only acetate is formed as a by-product. In this case, two molecules of $NADH_2$ are formed and, therefore, the PD yield amounts to 2/3 mol of PD per mole of glycerol consumed (without consideration of biomass formation). Generally, the more $NADH_2$ is available the more PD can be obtained.

Gottschalk and Averhoff (1990) as well as Tran Dinh and Hill (1989) investigated the effect of the cosubstrate glucose on PD formation by *Citrobacter freundii* DSM 30040 and *K. pneumoniae* DSM 4270, respectively, and reported increased PD yields based on glycerol consumption. However, as glucose and glycerol have similar prices the use of glucose as a hydrogen donor does not offer economical advantages.

The fermentation of glycerol by *K. pneumoniae* DSM 2026 (NCTC 418) proceeds with satisfactory yields and final PD concentrations (Homann et al. 1990; Tag 1990). This microorganism converts up to 120 $g \cdot l^{-1}$ glycerol, PD yields being 0.53 to 0.56 $mol \cdot mol^{-1}$ glycerol. The overall PD productivities achieved range from 1.68 to 2.2 $g \cdot l^{-1} \cdot h^{-1}$, respectively. The continuous cultivation of *K. pneumoniae* DSM 2026 has also been studied by Streekstra et al. (1987) and Tag (1990). However, in view of the pathogenic features of *K. pneumoniae* its application in industrial plants will be limited.

In this study, the conversion of glycerol to PD by *Clostridium butyricum* (DSM 5431) was investigated (Kretschmann et al. 1990). This strain has already been

Offprint requests to: W.-D. Deckwer

studied in continuous culture (pH auxostat) to detect growth inhibition due to substrate and products (Biebl 1991). The particular purpose of this paper was to discover the conditions that lead to high PD concentrations and productivity. In addition, the scale-up of the anaerobic conversion to pilot plant scale in different reactor types was studied in order to clarify whether or not there is any limitation for scaling up this process to industrial size.

Materials and methods

Bacterial strain. C. butyricum DSM 5431 was used in this study.

Media. The composition of the preculture medium per litre was: 3.4 g K_2HPO_4; 1.3 g KH_2PO_4; 2.0 g $(NH_4)_2SO_4$; 0.2 g $MgSO_4 \cdot 7H_2O$; 0.02 g $CaCl_2 \cdot 2H_2O$; 2.0 g $CaCO_3$; 1.0 g yeast extract; 20.0 g glycerol; 1.0 ml trace element solution; 2.0 ml Fe solution. The composition of the Fe solution per litre was: 5 g $FeSO_4 \cdot 7H_2O$; 4 ml HCl (37%).

The ingredients of the nutrient medium of the main culture per litre were: 1 g K_2HPO_4; 0.5 g KH_2PO_4; 5 g/100 g glycerol $(NH_4)_2SO_4$; 0.2 g $MgSO_4 \cdot 7H_2O$; 0.02 g $CaCl_2 \cdot 2H_2O$; 2.0 g $CaCO_3$; 1.0 g yeast extract; as required 20.0–50.0 g glycerol; 1.0 ml trace element solution; 1.0 ml Fe solution.

The trace element solution per litre consisted of: 70 mg $ZnCl_2$; 0.1 g $MnCl_2 \cdot 4H_2O$; 60 mg H_3BO_3; 0.2 g $CoCl_2 \cdot 2H_2O$; 20 mg $CuCl_2 \cdot 2H_2O$; 25 mg $NiCl_2 \cdot 6H_2O$; 35 mg $Na_2MoO_4 \cdot 2H_2O$; 0.9 ml HCl (37%).

Experimental set-up. Tables 1 and 2 give the major dimensions of the bioreactors used in this study. All reactors were equipped with controls for temperature, pH, agitation speed and aeration rate. In fed-batch cultivations the glycerol was sterilized separately in a reservoir vessel and fed to the reactor by a sterile supply line. The smallest STR (KLF 2000, Bioengineering, Wald, Switzerland) was used to optimize temperature and pH. The other reactors given in Tables 1 and 2 were all from B. Braun Diessel (Melsungen, FRG). The effect of stirrer speed and aeration rate was determined in a stirred-tank reactor (STR 42).

Reactor comparison studies were carried out in ALR 50 and STR 42 both with the same working volume. Both the ALR and STR were inoculated simultaneously with the same preculture grown in a 10-l reactor and harvested in the exponential growth phase. The air-lift reactors (ALR 50 and 1500) are designed similarly with regard to their geometrical sizes, i.e. $h_L/d_R = 10$ and $d_I/d_R = 0.71$ (see Table 1). The top sections of the air-lift reactors are enlarged to facilitate bubble break-up and foam destruction.

Analytical methods. Fermentation products were determined by gas chromatography (wide bore column HP 17 (cross-linked) of 10 m length, installed in a Hewlett Packard (HPI, Frankfurt/M, FRG) instrument (model HP 5890 A) with a flame ionisation detector and connected with an automatic sampling device (model HP 7673)). The column temperature was programmed from 40° C to 230° C in two steps. The carrier gas and the internal standard used were helium and *n*-propanol, respectively.

Glycerol analysis was done enzymatically using the test kit of Boehringer (Mannheim, FRG). Optical density was measured at 650 nm in a spectral photometer (Pharmacia-LKB, Uppsala, Sweden). Cell concentrations were measured as dry weight (DW) and were obtained from washed centrifuged pellets dried at 40° C for 48 h in vacuum.

Culture methods. Inocula for the bioreactor were prepared in 100 ml flasks containing 50 ml deoxygenated preculture medium. The flasks were incubated at 30° C for 16 h and subsequently transferred to the KLF 2000 bioreactor in amounts of 10% by vol. Bioreactors with a working volume larger than 10 l were inoculated with 10% precultures by vol. harvested in the exponential growth phase. The cultivations were carried out at 35° C and pH 7.0 if not otherwise stated. The pH was controlled by addition of 4 M KOH. Aeration with nitrogen (0.05 or 0.1 vvm) provided anaerobic conditions. The antifoam used was Desmophen (Bayer AG, Leverkusen, FRG).

Table 1. Air-lift reactors (ALR) used

Reactor data	Air-lift reactor	
	ALR 50	ALR 1500
Total volume, V_R (m^3)	0.050	1.500
Working volume, V_L (m^3)	0.030	1.200
Reactor diameter, d_R (m)	0.150	0.500
Draft tube diameter, d_I (m)	0.106	0.355
Top section diameter, d_t (m)	0.300	0.800
Height of reactor, h_R (m)	1.800	6.000
Height of liquid, h_L (m)	1.500	5.000
d_I/d_R	0.710	0.710
h_L/d_R	10.000	10.000

Results and discussion

Optimization

Cultivation results of anaerobic processes are affected by various influences, i.e., temperature, pH, and concentrations of substrate and products. To determine such influences batch cultivations were carried out in the 1.4-l reactor (KLF 2000). The results are given in Tables 3 and 4. From Table 3 it is seen that complete glycerol consumption and the highest PD productivity

Table 2. Stirred-tank reactors (STR) used

Reactor data	KLF 2000	STR 10	STR 42	STR 300	STR 3000
Total volume, V_R (m^3)	$2.0 \cdot 10^{-3}$	0.014	0.042	0.30	3.00
Working volume, V_L (m^3)	$1.4 \cdot 10^{-3}$	0.010	0.030	0.20	2.00
Reactor diameter, d_R (m)	0.095	0.215	0.260	0.50	1.28
Stirrer diameter, d_S (m)	0.040	0.120	0.100	0.20	0.43
Height of reactor, h_R (m)	0.300	0.542	0.780	1.60	2.57
Height of liquid, h_L (m)	0.210	0.330	0.600	1.06	1.70
d_S/d_R	0.421	0.560	0.390	0.40	0.34
h_L/d_R	2.211	1.530	2.300	2.12	2.33

Table 3. Batch fermentation of 2% glycerol at different temperatures ($V_L = 1.4$ l; pH = 7.0)

T (° C)	Glycerol consumed, c_S (g·l^{-1})	Products formed, c (g·l^{-1})				Q_{PD} (g·l^{-1}·h^{-1})
		1,3-PD	Acetate	Butyrate	Ethanol	
30.0	16.5	5.70	0.90	2.00	0.40	0.48
32.0	20.0	8.60	1.56	1.33	0.23	0.86
33.5	17.3	8.48	1.11	2.28	0.42	0.94
35.0	20.0	10.10	1.47	1.72	0.21	1.13
37.0	18.0	7.95	0.27	2.66	0.43	0.61

1,3-PD, 1,3-propanediol; Q_{PD}, PD productivity

Table 4. Batch fermentation of 5% glycerol at different pH ($V_L = 1.4$ l; $T = 35°$ C)

pH	Glycerol consumed, c_S (g·l^{-1})	Products formed, c (g·l^{-1})				Q_{PD} (g·l^{-1}·h^{-1})
		1,3-PD	Acetate	Butyrate	Ethanol	
6.0	43.0	21.1	1.95	4.41	0.15	1.6
6.6	45.0	23.5	3.51	3.43	0.27	2.2
6.8	43.0	25.0	3.36	3.12	0.13	2.2
7.0	48.0	23.2	3.05	2.98	0.20	2.2
7.2	45.0	22.7	3.02	3.25	0.13	2.0
7.4	44.0	23.4	2.89	3.48	0.13	2.0
8.0	30.0	18.6	1.36	2.54	0.07	0.7

was obtained at $T = 35°$ C. At this temperature the effect of pH was studied. The best results were found in the range of pH 6.6–7.0. Therefore, all further cultivations were carried out at $T = 35°$ C and pH 7.0, the product yield being around 0.6 mol PD·mol^{-1} glycerol consumed. Figure 1 shows a typical run performed under such conditions. The by-products generated were acetate and butyrate (Fig. 1, Tables 3 and 4). The formation of ethanol was negligible.

The influence of glycerol concentration on growth and product formation was examined in several batch experiments carried out in the STR 10. With increasing concentration of substrate, growth was inhibited. This result is in accordance with the findings of Biebl (1991). To achieve glycerol consumption in quantities up to 100 g·l^{-1}, cultivations were carried out in fed-batch operation, starting with initial glycerol concentrations (C_0^S) of either 20 g·l^{-1} or 50 g·l^{-1}. The course of such a fed-batch cultivation is shown in Fig. 2. After about 6 h the initial glycerol was consumed and additional glycerol was fed to the reactors in amounts giving again a concentration of about 20 g·l^{-1} in the reactor. This could be done four times giving a final PD concentration of around 50 g·l^{-1} at an overall productivity of 2.76 g·l^{-1}·h^{-1}. The time of complete glycerol consumption was accompanied by a sudden rise in the pH. This was used as a signal to start glycerol feeding.

The effect of stirrer speed (N) and aeration rate ($\dot{V}_G$) on growth and product formation was investigated in the STR 42 ($V_L = 30$ l). As shown in Fig. 3 for fed-batch cultivations nitrogen aeration rate (0.05 and 0.1 vvm) and agitation speed (150–475 min^{-1}) had no significant effects. As expected, mass transfer and mixing processes did not influence reactor performance. However,

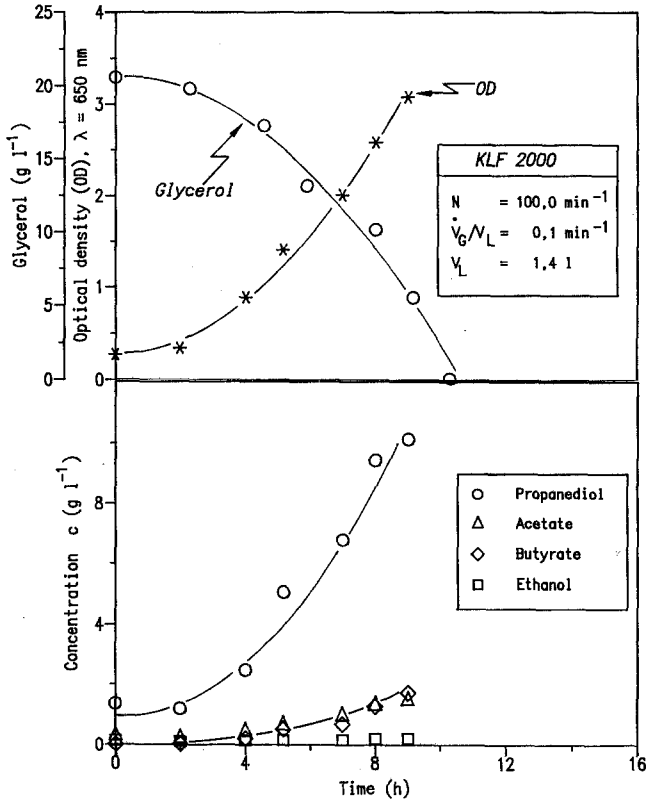

Fig. 1. Batch fermentation of 2% glycerol by *Clostridium butyricum* DSM 5431 at 35° C and pH 7.0: N, stirrer speed; $\dot{V}_G$, aeration rate; V_L, working volume

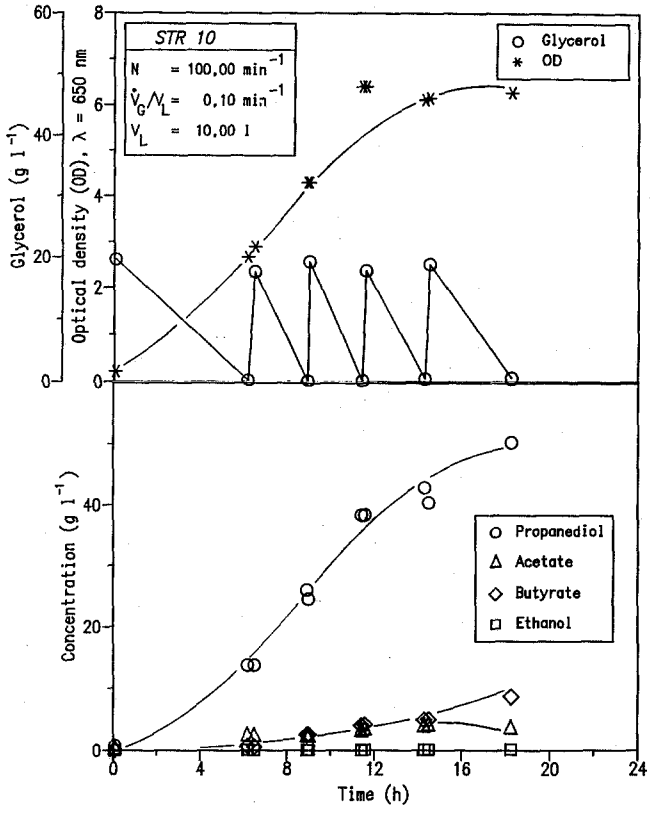

Fig. 2. Fed batch fermentation of glycerol by *C. butyricum* at 35° C and pH 7.0: initial glycerol concentration ($c_S^0 = 20\ g \cdot l^{-1}$; $V_L = 10\ l$)

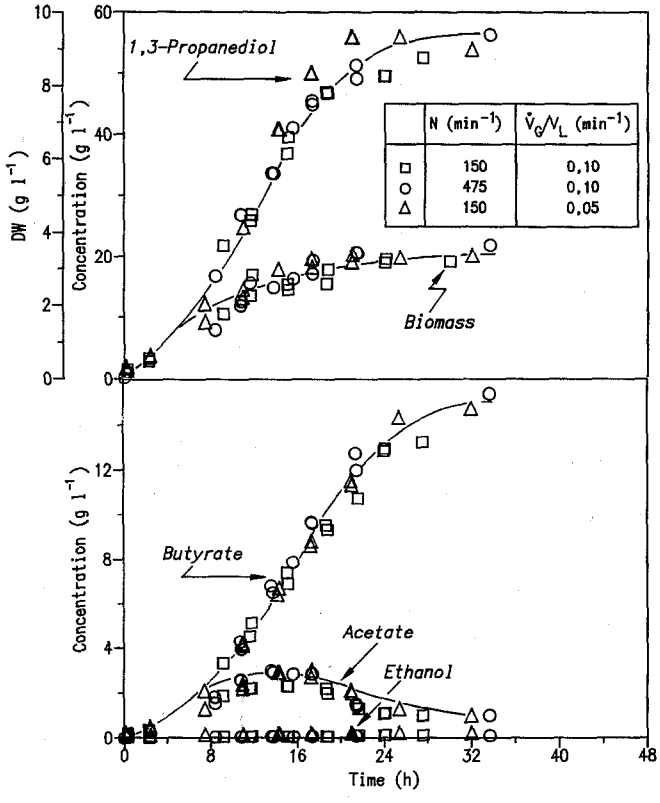

Fig. 3. Formation of biomass (DW, dry weight) and products in stirred-tank reactor (STR) 42 at different stirrer speeds and aeration rates: fed batch fermentation; $c_S^0 = 50\ g \cdot l^{-1}$; $V_L = 30\ l$; $T = 35°\ C$; pH 7.0

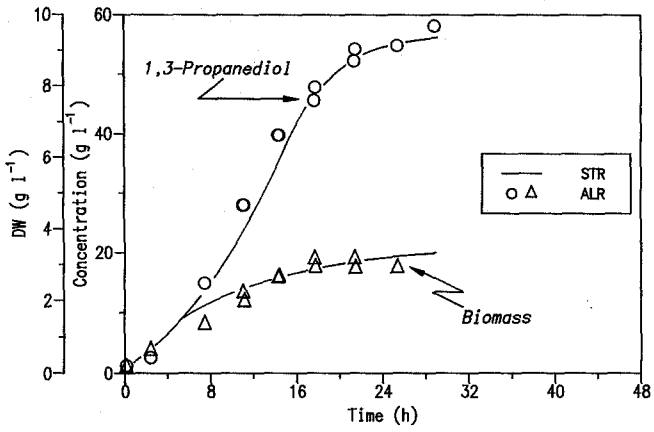

Fig. 4. Comparative cultivation in STR and air-lift reactor (ALR): fed-batch cultivation; $c_S^0 = 50\ g \cdot l^{-1}$; $V_L = 30\ l$; $T = 35°\ C$; pH 7.0, 0.05 vvm

as compared to no aeration, desorption of CO_2 was greatly enhanced when applying a low nitrogen flow rate. Thus, consumption of KOH could be reduced drastically. The overall productivities in the 30 l reactor were similar the those obtained in smaller equipment (i.e., 2.3-2.7 $g \cdot l^{-1} \cdot h^{-1}$). The shape of the acetate concentration curve suggests that acetate was consumed in the second part of the cultivation, i.e., when the PD concentration curve passed its inflection point. In contrast to acetate butyrate accumulated in the culture medium up to concentrations of 14 $g \cdot l^{-1}$.

Reactor comparison

Comparative fed-batch cultivations were performed in the STR and ALR of 30 l volume under optimal conditions ($T = 35°\ C$, pH 7.0, 0.05 vvm). As depicted in Fig. 4, no differences in formation of biomass and PD were observed. About 100 $g \cdot l^{-1}$ glycerol were converted to approximately 55 $g \cdot l^{-1}$ PD (0.66 $mol \cdot mol^{-1}$ glycerol), starting with an initial glycerol concentration of 50 $g \cdot l^{-1}$. Therefore, both the ALR and STR can equally be used to ferment glycerol to PD by *C. butyricum*. The amount of antifoam agent that had to be used during fermentation in the STR was 3 $ml \cdot l^{-1}$ culture medium whereas foaming in the ALR could be depressed by using only 1 $ml \cdot l^{-1}$. Obviously, stirring the culture medium gives rise to excessive foam formation while foaming in the ALR was only moderate and caused no problems.

Scale-up

The scale-up of glycerol fermentation by *C. butyricum* was studied in ALR and STR. The ALR 50 and 1500 with working volumes of 30 and 1200 l, respectively, were used, the volumetric scale-up factor being 40. The concentrations of biomass and PD are given in Fig. 5. The slight differences observed for the two reactors does not represent a scale-up effect but are a result of

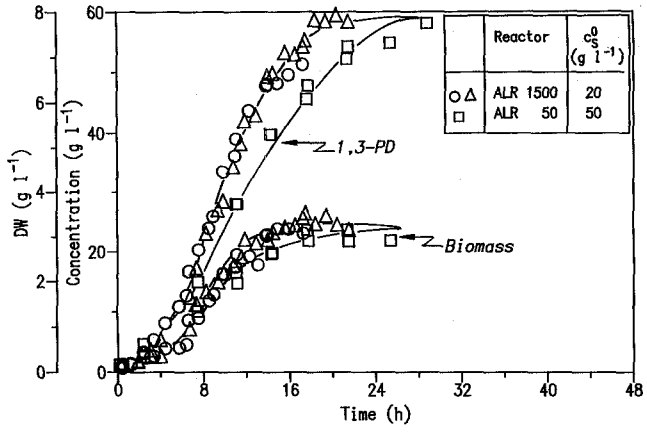

Fig. 5. Scale up of 1,3-propanediol (1,3-PD) production in ALR: fed-batch fermentation; ALR 50: $c_S^0 = 50\ g \cdot l^{-1}$; ALR 1500: $c_S^0 = 20\ g \cdot l^{-1}$

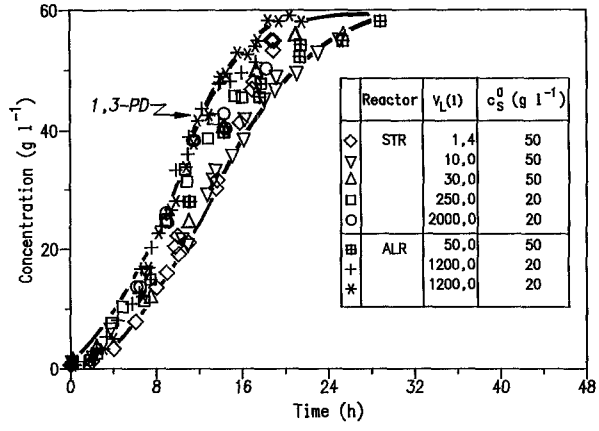

Fig. 6. Comparison of scale-up experiments in the STR and ALR

different initial concentrations of glycerol, as proved by additional cultivations. The effect of glycerol inhibition was not very pronounced (Biebl 1991) but increased the cultivation time and hence reduced PD productivity while the final product concentrations did not differ. As pointed out above, foaming in the ALR was not serious and destruction of foam was additionally supported by using a nozzle for spraying the glycerol feed and KOH solution from the reactor top onto the foam layer.

Production of PD was also studied in the STR 300 and 3000 with working volumes of 250 and 2000 l, respectively. Only gentle mixing was applied, the energy input being in the range 60–80 $W \cdot m^{-3}$. The results are shown in Fig. 6. Here the PD concentration is given for all reactors used at the two initial glycerol concentrations applied. As already pointed out the differences in these fed-batch fermentations were due to the differing starting concentrations. Again any influence of reactor type cannot be recognized even for the larger volumes studied. As summarized in Table 5, the final product concentrations and the productivities achievable ranged from 50 to 58 $g \cdot l^{-1}$ and 2.3 to 2.9 $g \cdot l^{-1} \cdot h^{-1}$, respectively. As ALR require less investment and lower operation costs, microbial production of PD in this reactor type is more attractive from the economical point of view than the use of stirred reactors. On the basis of the findings of this study it is concluded that the scale-up of this microbial conversion to industrial reactors ($V_R \geq 10\ m^3$) will not cause additional difficulties.

References

Biebl H (1991) Use of pH auxostat for the measurement of product inhibition. Appl Microbiol Biotechnol, in press

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

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

Gottschalk G, Averhoff B (1990) Process for the microbial preparation of 1,3-propanediol from glycerol. European patent no. EP 0373230 A1

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

Table 5. Fed-batch fermentation of glycerol up to a scale of 2.0 m^3 ($T = 35°C$; pH 7.0; 0.05 vvm)

V_L (l)	Glycerol, c ($g \cdot l^{-1}$)		Products formed, c ($g \cdot l^{-1}$)				Q_{PD} ($g \cdot l^{-1} \cdot h^{-1}$)
	C_S^0	consumed	1,3-PD	Acetate	Butyrate	EtOH	
1.4[a]	50.8	108.4	54.9	1.97	11.60	0.18	2.40
10[a]	56.5	96.8	47.8	5.67	7.04	0.14	2.30
30[a]	47.6	107.0	56.0	1.90	14.60	0.11	2.65
200[a]	44.5	103.7	46.1	0.83	13.20	0.28	2.43
250[a]	51.2	84.7	45.5	2.10	10.32	0.07	2.84
2000[a]	20.0	91.3	50.3	7.02	8.71	0.17	2.76
30[b]	48.9	103.0	58.0	1.40	10.91	0.15	2.70
1200[b]	21.0	88.8	51.3	5.91	6.37	0.11	2.97
1200[b]	22.9	96.8	58.0	5.77	9.29	0.09	2.70

C_S^0, initial glycerol concentration
[a] STR
[b] ALR

Kretschmann J, Carduck F-J, Deckwer W-D, Tag C, Biebl H (1990) Fermentative Herstellung von 1,3-Propandiol. European patent no. EP 0361082 A2

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

Ruch FE, Lin EEC (1975) Independent constitutive expression of the aerobic and anaerobic pathways of glycerol metabolism in *Klebsiella aerogenes*. J Bacteriol 124:348

Schütz H, Radler F (1984) Anaerobic reduction of glycerol to propanediol-1,3 by *Lactobacillus brevis* and *Lactobacillus buchneri*. Syst Appl Microbiol 5:169

Streekstra H, Teixeira de Mattos MJ, Neijssel OM, Tempest DW (1987) Overflow metabolism during anaerobic growth of *Klebsiella aerogenes* NCTC 418 on glycerol and dihydroxyacetone in chemostat culture. Arch Microbiol 147:268–275

Tag C (1990) Mikrobielle Herstellung von 1,3-Propandiol. PhD Dissertation, University of Oldenburg, FRG

Tran-Dinh K, Hill FF (1989) Verfahren zur Herstellung von 1,3-Propandiol. German patent no. DE 3734764 A1