Appl Microbiol Biotechnol (2002) 59:303–309
DOI 10.1007/s00253-002-1009-5

ORIGINAL PAPER

A. Converti · P. Perego

Use of carbon and energy balances in the study of the anaerobic metabolism of *Enterobacter aerogenes* at variable starting glucose concentrations

Received: 7 January 2002 / Revised: 18 March 2002 / Accepted: 22 March 2002 / Published online: 3 May 2002

Abstract The anaerobic metabolism of *Enterobacter aerogenes* was studied in batch culture at increasing initial glucose levels ($9.0<S_0<72$ g l^{-1}). The ultimate concentrations of fermentation products were utilized to check a metabolic flux analysis based on simple carbon mass and energy balances that promise to be suitable for the study of different fermentation processes, either under aerobic or anaerobic conditions. The stoichiometric coefficients of products collected at increasing starting glucose concentrations under anaerobic conditions suggest: (a) little influence of starting glucose level on the formation of the main fermentation products (2,3-butanediol and ethanol); (b) possible inhibition of 2,3-butanediol and lactate formations by increased ethanol concentration; (c) consequent increase in carbon flux through the remaining metabolic pathways with increased molar productions of succinate, acetate and hydrogen; (d) relative constancy of the molar production of ATP and CO_2.

Introduction

Enterobacter aerogenes is a facultative anaerobe that, depending on the strain, produces preferentially formate or hydrogen under anaerobic conditions (Kosaric and Lying 1988; Perego et al. 1998; Tanisho and Ishiwata 1995; Tanisho et al. 1998; Zajic et al. 1987); however, when fermentation is stimulated by microaeration this bacterium produces 2,3-butanediol (Converti and Perego, submitted; Zeng and Deckwer 1991, 1992; Zeng et al. 1990a, b, 1994).

Butanediol is a very interesting compound employed as solvent, drug carrier, or for use as an intermediate in the chemical industry or in the manufacture of polymers, ink, fumigants, perfumes, moistening and softening agents, explosives and plasticizers (Magee and Kosaric 1987). In addition, butanediol can be transformed, by simple dehydration, into the industrial solvent methyl-ethyl ketone (Emerson et al. 1982) or, by further dehydration, into 1,3-butadiene, that can be used for rubber synthesis or dimerized to styrene (Jansen et al. 1984).

Several authors have studied glucose metabolism in *E. aerogenes*, varying the operative conditions in order to favor one or another of the various fermentation products (Garg and Jain 1995; Maddox 1988; Magee and Kosaric 1987; Perego et al. 1998).

Butanediol is produced by *E. aerogenes* under oxygen-limited and anaerobic conditions (Kosaric and Lyng 1988). Due to the low oxygen availability, the excess reducing power deriving from glycolysis cannot be totally regenerated by the respiratory chain; therefore, this fermentation is always connected with other $NADH_2^+$-consuming pathways, leading to the formation of several products (butanediol, acetoin, lactate, acetate, ethanol, succinate, etc.). The complexity of such a microbial system makes modeling very difficult, and there have been only a few attempts to perform a stoichiometric study of its metabolism (Zeng and Deckwer 1992; Zeng et al. 1990a).

Several studies have demonstrated that oxygen uptake rate is the most crucial parameter (Converti and Perego, submitted; de Mas et al. 1988; Jansen et al. 1984; Ramachandran and Goma 1987). In the absence of air, growth was slow (Sablayrolles and Goma 1984) and most of the carbon was converted into ethanol, formate, butanediol, acetoin and acetate (Maddox 1988). On the other hand, hydrogen was the main fermentation product of *E. aerogenes* strains provided with strong formate-hydrogen lyase activity (Kosaric and Lyng 1988; Perego et al. 1998; Tanisho and Ishiwata 1994) or the so-called NADH pathway (Tanisho and Ishiwata 1995; Tanisho et al. 1998). Oxygen-limited conditions suppressed the formation of ethanol and formate and stimulated the production of butanediol, acetoin, acetate, cell mass, and carbon dioxide (Voloch et al. 1985), while both butanediol and acetoin production was strongly affected by excess oxygen, because an increasing fraction of carbon source was utilized for growth (Jansen et al. 1984).

A. Converti (✉) · P. Perego
Department of Chemical and Process Engineering "G.B. Bonino", University of Genoa, via Opera Pia 15, 16145 Genoa, Italy
e-mail: converti@unige.it
Tel.: +39-010-3532593, Fax: +39-010-3532586

Less is known about the effects of the type and starting level of the carbon source (Magee and Kosaric 1987). Sablayrolles and Goma (1984) observed that 2,3-butanediol yield by *Aerobacter aerogenes* increased by 40% and the specific growth rate decreased from 0.66 to 0.19 h^{-1} when the starting glucose concentration was increased from 22 to 195 g l^{-1}. Qualitatively similar effects were observed by Jansen et al. (1984) for the same microbial system grown on xylose at starting substrate levels (S_0) $5.0<S_0<150$ g l^{-1}, but the butanediol yields were lower, in particular at the lowest substrate level. Since the results with *Bacillus polymyxa* showed an opposite trend, it was evident that the influence of carbon source type and concentration is species-dependent (Magee and Kosaric 1987).

To study the progressive shift of *E. aerogenes* metabolism from anaerobic fermentation to microaerobic butanediol production and finally to respiration, we carried out a preliminary batch study at S_0=18 g l^{-1}, increasing the specific oxygen uptake rate up to 72.7 $mmol_{O2}$ $C\text{-}mol_{DW}^{-1}$ h^{-1} (Converti and Perego, submitted). The ultimate concentrations of products were utilized to check a general model based on carbon mass and energy balances that can be applied to different batch fermentations, either under aerobic or anaerobic conditions. Jansen et al. (1984) used similar carbon balances to study the influence of variable oxygen uptake rate and starting substrate level on xylose uptake by *Klebsiella oxytoca*. However, the final carbon recoveries were limited to the production of biomass and main fermentation products (butanediol, carbon dioxide, ethanol and acetate), thus providing a simplified, albeit clear picture of this type of metabolism. Such an approach is used here to elucidate the metabolism of *E. aerogenes* in batch culture at variable starting glucose level.

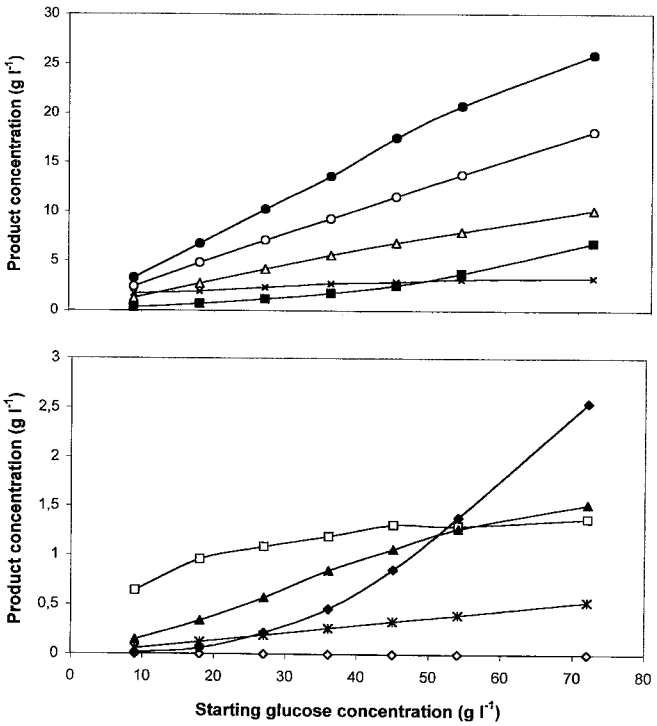

Fig. 1 Experimental data of batch anaerobic fermentations by *Enterobacter aerogenes* at variable starting glucose levels. Product concentrations (g l^{-1}): ○ Butanediol, ● CO_2, △ ethanol, × biomass, ■ succinate, □ lactate, ◇ formate, ▲ acetoin, ◆ acetate, ✳ hydrogen

Materials and methods

Microorganism

Cultures of *E. aerogenes* (NCIMB 10102) were obtained by periodically recovering the cells from batch runs. Pure cultures were maintained on nutrient agar slants at 4 °C. The cells were then incubated aerobically, under optimal conditions previously selected for this microbial system (pH=6.0 and T=39 °C; Perego et al. 2000), in shaken flasks on a rotary shaker and harvested at the end of the exponential phase. The pre-culture medium (nutrient broth containing 2.0 g yeast extract l^{-1}, 5.0 g peptone l^{-1}, 5.0 g NaCl l^{-1}, and 1.0 g beef extract l^{-1}) was sterilized at 120 °C for 15 min and supplemented with glucose at the same concentrations as used for fermentations. The cells were grown for 48 h, centrifuged and aseptically inoculated into the fermenter.

Analytical methods

Butanediol, acetoin, hydrogen, acetate, succinate, lactate, formate and ethanol concentrations were measured by gas chromatography. After passing the exit gas through suitable concentration of $Ba(OH)_2$ absorbing solutions, carbon dioxide production was determined by a titrimetric method (ISO 1997) and expressed as g per liter of reactor volume.

Glucose concentration was determined using an HPLC Hewlett Packard 1100 equipped with IR-detector and Hypersil 200×4.6 mm column kept at 35 °C. An 80:20 acetonitrile/water solution was used as mobile phase at a flow rate of 1.0 ml min^{-1}.

Cell concentration was determined by filtering a known volume of culture broth through 0.2-μm autoclavable membrane filters. The filters were dried at 105 °C until no weight change between consecutive measurements was observed. A few ml of a concentrated cell suspension were added to the medium up to the desired starting biomass level (X_0=1.34 g_{DW} l^{-1}).

Operating conditions

A 7-l Setric 7F bioreactor with a 5.0-l working volume, stirred at 150 rpm, was employed for batch fermentations. The bioreactor was equipped with pH, pO_2, temperature, antifoam, dissolved oxygen, and weight controls. The pH of the fermentation broth was automatically regulated at 6.0±0.1 pH units with a Setric PAP 7F peristaltic pump, which injected a fine stream of 30% NaOH solution.

Sparging the reactor with nitrogen ensured anaerobic conditions. Two flow-meters were used to control the gas flow into and from the reactor. The exit gas from the reactor was dried prior to CO_2 absorption. The fermentor was sterilized by autoclaving at 120 °C for 15 min.

Carbon mass end energy balances

Batch fermentations were carried out up to total carbon consumption to evaluate and quantify the effect of starting glucose concentration on the anaerobic metabolism of *E. aerogenes*. Carbon mass and energy balances, presented in the Appendix for the consumption of 1.0 mol glucose, refer to the general microbial metabolism

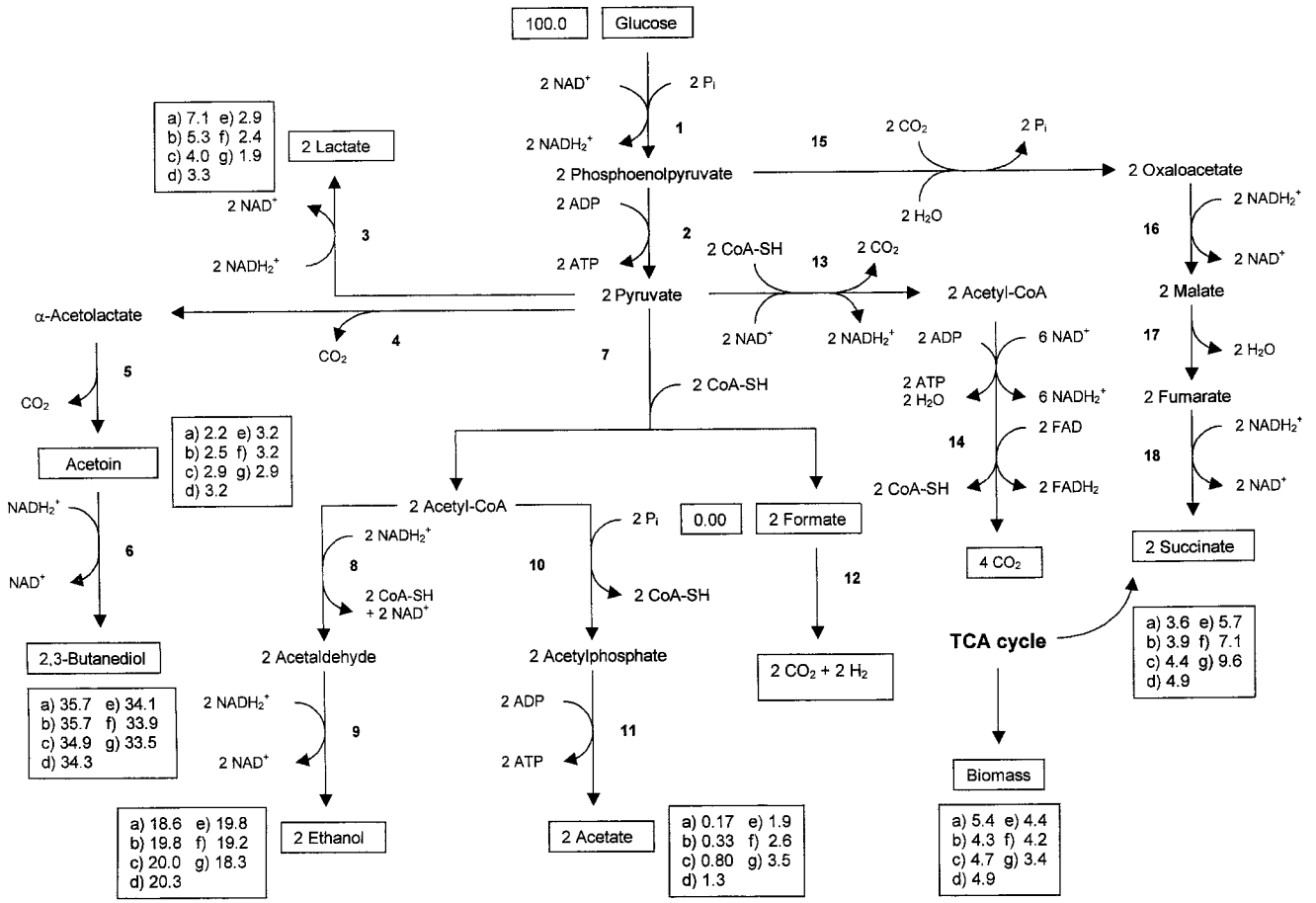

Fig. 2 Experimental flux patterns for the anaerobic metabolism of *E. aerogenes*. All fluxes are given in C-mol carbon transferred and are referred to the consumption of 100 C-mol glucose. S_0 (g l^{-1}): *a* 9.0, *b* 18, *c* 27, *d* 36, *e* 45, *f* 54, *g* 72. Enzymes: *1* Enzymes of glycolysis, *2* pyruvate kinase, *3* lactate dehydrogenase, *4* acetolactate synthase, *5* acetolactate decarboxylase, *6* butanediol dehydrogenase, *7* pyruvate-formate lyase, *8* acetaldehyde dehydrogenase, *9* alcohol dehydrogenase, *10* phospho-transacetylase, *11* acetate kinase, *12* formate-hydrogen lyase, *13* pyruvate dehydrogenase, *14* enzymes of TCA cycle, *15* phosphoenolpyruvate carboxylase, *16* malate dehydrogenase, *17* fumarase, *18* succinate dehydrogenase

based on mixed acid fermentation. These were carried out using the experimental final concentrations of products (butanediol, ethanol, biomass, acetate, lactate, succinate, formate, carbon dioxide, hydrogen, acetoin and biomass) and are based on the following assumptions: (a) the reducing power associated with precursor formation is negligible, according to the stoichiometry presented by Roels (1983); (b) the carbon fraction consumed for growth (6ζ) can be estimated assuming the average dry biomass composition reported by Roels (1983) for *Klebsiella aerogenes* ($CH_{1.74}$ $O_{0.43}$ $N_{0.23}$) and neglecting, according to Zeng et al. (1990c), the amount of CO_2 produced in biomass synthesis; (c) the sum of mol acetate and mol ethanol due to pyruvate-formate lyase activity corresponds to the mol formate before its transformation into CO_2 and H_2.

Results

Figure 1 shows the experimental data collected at the end of batch anaerobic fermentations with varying starting glucose concentrations from 9.0 to 72 g l^{-1}. The molar yields of these products per mol substrate consumed for fermentation are presented in Table 1, while the flux patterns for anaerobic metabolism are illustrated in Fig. 2, where all fluxes are given in C-mol carbon transferred and refer to the consumption of 100 C-mol glucose. Similar work was performed by Zeng and Deckwer (1992) in continuous culture to evaluate the effects of oxygen uptake rate and agitation speed on product formation rates and relative utilization of the TCA cycle.

From the results of Table 1 and Fig. 1 it should be noted that an increase in the starting concentration of the carbon source was responsible for poor effects on the formation of the main fermentation products (2,3-butanediol, ethanol and carbon dioxide) and remarkable variation in the relative proportions of the other products. In particular, (a) molar production of 2,3-butanediol (α) was hardly affected, decreasing only from 0.53–0.54 to 0.50 mol butanediol mol_G^{-1}; (b) although acetoin yield (β) was about one order of magnitude less than that of butanediol, it was much more sensitive to S_0 variations, progressively increasing from 0.033 up to 0.048 mol acetoin mol_G^{-1} for $36<S_0<54$ g l^{-1}, but decreasing beyond this threshold; (c) molar production of hydrogen (γ_1) and ethanol (γ_2) suddenly increased from 0.56 to 0.65–0.66 mol hydrogen mol_G^{-1} and from 0.56 to 0.61 mol ethanol mol_G^{-1}, respectively, with increasing S_0 up to 36 g l^{-1}, whereas above that glucose level threshold

Table 1 Stoichiometric coefficients and products molar yields (mol mol_G^{-1}) of batch anaerobic fermentations of glucose by *E. aerogenes*, determined at variable starting glucose levels, S_0. Data refer to the consumption of 1 mol glucose. X_0=1.34 g_{DW} l^{-1}

S_0 (g l^{-1})	9.0	18	27	36	45	54	72
Experimental coefficients							
α	0.53	0.54	0.52	0.51	0.51	0.51	0.50
β	0.033	0.038	0.043	0.048	0.048	0.048	0.043
γ	0.28	0.30	0.31	0.32	0.32	0.33	0.33
γ_1	0.56	0.60	0.62	0.65	0.65	0.65	0.66
γ_2	0.56	0.59	0.60	0.61	0.59	0.57	0.55
γ_3	0.005	0.010	0.024	0.038	0.057	0.077	0.106
δ	0.071	0.053	0.040	0.033	0.029	0.024	0.019
ε	0.027	0.029	0.033	0.037	0.043	0.053	0.072
ζ	0.054	0.043	0.047	0.049	0.044	0.042	0.034
Experimental yields							
$Y_{ATP/G}$	1.95	1.95	1.96	1.96	1.97	1.97	1.96
$Y_{CO2/G}$	1.50	1.53	1.55	1.54	1.59	1.57	1.47
$Y_{X/G}$	0.32	0.26	0.28	0.29	0.26	0.25	0.21
$Y_{X/ATP}$	0.16	0.13	0.14	0.15	0.13	0.13	0.10
Theoretical yields							
[a]$Y_{CO2/G}$	1.64	1.69	1.69	1.70	1.68	1.66	1.60
[b]$Y_{CO2/G}$	1.64	1.69	1.70	1.68	1.68	1.65	1.61

[a] Values calculated by the stoichiometric coefficients of the Appendix
[b] Values calculated as difference between C-mol glucose and C-mol products

the former remained nearly constant while the latter decreased; (d) biomass grew very slowly, reaching a final concentration of only 1.73–3.29 g_{DW} l^{-1}; (e) molar productions of acetate (γ_3) and succinate (2ε) quickly grew with S_0 increasing from 0.005 to 0.11 mol acetate mol_G^{-1} and from 0.027 to 0.072 mol succinate mol_G^{-1}, respectively; (f) molar production of lactate (2δ) was strongly affected by S_0, decreasing from 0.071 to 0.019 mol lactate mol_G^{-1}.

Discussion

Taken together, these results demonstrate that, under anaerobic conditions, most of the starting carbon was consumed to produce 2,3-butanediol (35%), ethanol (19%) and carbon dioxide (28%), while the yields of acetoin, lactate, succinate and biomass were one order of magnitude less. On the other hand, no formate accumulation was detected (2γ-γ_1=0).

The *E. aerogenes* strain used in this work was already shown to be a satisfactory producer of hydrogen under strict anaerobic conditions (Perego et al. 1998). As recently demonstrated by the low total amount of acetate and ethanol in continuous anaerobic cultivation of strains able to overproduce hydrogen (Tanisho and Ishiwata 1995), hydrogen evolution is no longer considered to be linked only to the formate pathway, but also to the so-called NADH pathway (Tanisho et al. 1989). According to these findings, the satisfactory butanediol yields (Table 1) with respect to previous work (Perego et al. 1998) were likely due to the disappearance of NADH dehydrogenase activity, because of the progressive adaptation of our microorganism to microaerobiosis (Perego et al. 2000).

Since there is no evidence in the literature either of glucose induction of the formate-hydrogen lyase complex or of substrate inhibition of butanediol dehydrogenase, the reason for the changes in ethanol, hydrogen, acetate, and butanediol formation should alternatively be seen in the inhibition caused by product accumulation, in particular ethanol, which is a strong inhibitor in Enterobacteria and may be active even at the concentrations observed in this work. In fact, Zeng and Deckwert (1991) demonstrated that the specific growth rate of *E. aerogenes* decreased linearly with the addition of ethanol, was reduced to 50% at an ethanol concentration of 20 g l^{-1}, and completely stopped at 45 g l^{-1}. A possible ethanol inhibition of lactate dehydrogenase activity is also suggested in Fig. 2 by the progressive decrease in the percentage of carbon consumed for lactate production. This product inhibition of butanediol and lactate productions would be responsible for an increased flux of carbon towards the remaining metabolic pathways, mainly leading to the formations of acetate and hydrogen.

The production of succinate under anaerobic conditions is consistent with the existence of the anaplerotic reaction catalyzed by phosphoenolpyruvate carboxylase, which is necessary to satisfy the redox balance of the main reductive metabolism of most Enterobacteriaceae (Magee and Kosaric 1987). Both increased formation of acetate and decreased lactate formation would be responsible for increased production of reducing equivalents, which could be regenerated by increased production of succinate. The changes might again be trigged by an inhibitory concentration of ethanol, forcing metabolism to form less toxic products. As a general rule, whenever a primary metabolic route tending to accumulate reducing power in the form of $NADH_2^+$ – such as the 2,3-butanediol pathway – is inhibited, regeneration of this cofactor is guaranteed by the stimulation of alternative pathways implying a net consumption of $NADH_2^+$, among which that leading to succinate (2ε) as the main product. This would justify the general accumulation of byproducts in fermentations carried out under suboptimal conditions.

The validity of the present approach was confirmed by checking the carbon material balance (Table 2) and

Table 2 Carbon material balance of batch anaerobic fermentations of 1 mol glucose by *E. aerogenes*, carried out at variable starting glucose levels, S_o. Values are given in C-mol

S_o (g l^{-1})	9.0	18	27	36	45	54	72
Reactants							
Glucose	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Products							
Butanediol	2.14	2.14	2.10	2.06	2.04	2.03	2.01
Acetoin	0.13	0.15	0.17	0.19	0.19	0.19	0.17
Ethanol	1.12	1.19	1.20	1.22	1.19	1.15	1.10
Lactate	0.43	0.32	0.24	0.20	0.17	0.14	0.11
Acetate	0.01	0.02	0.05	0.08	0.11	0.15	0.21
Succinate	0.22	0.23	0.26	0.30	0.34	0.42	0.58
Biomass	0.32	0.26	0.28	0.29	0.26	0.25	0.20
Carbon dioxide	1.50	1.53	1.55	1.54	1.59	1.57	1.47
Total products	5.87	5.84	5.85	5.88	5.89	5.90	5.87
Error (%)	–2.2	–2.7	–2.5	–2.0	–1.8	–1.7	–2.5

Table 3 Reduction degree balance of batch anaerobic fermentations of 1 mol glucose by *E. aerogenes*, carried out at variable starting glucose levels, S_o

S_o (g l^{-1})	9.0	18	27	36	45	54	72
Reactants							
Glucose	24.00	24.00	24.00	24.00	24.00	24.00	24.00
Products							
Butanediol	11.77	11.79	11.53	11.31	11.24	11.18	11.04
Acetoin	0.66	0.76	0.86	0.96	0.96	0.96	0.86
Ethanol	6.70	7.13	7.21	7.31	7.12	6.90	6.60
Lactate	1.70	1.27	0.96	0.79	0.70	0.46	0.46
Acetate	0.04	0.08	0.19	0.30	0.46	0.62	0.85
Succinate	0.76	0.81	0.92	1.04	1.20	1.48	2.02
Hydrogen	1.12	1.21	1.25	1.29	1.30	1.30	1.25
Oxygen	–0.28	–0.23	–0.25	–0.26	–0.23	–0.22	–0.25
Biomass	1.36	1.08	1.18	1.23	1.11	1.06	1.18
Total products	23.83	23.90	23.85	23.97	23.86	23.74	24.01
Error (%)	–0.71	–0.42	–0.62	–0.12	–0.58	–1.08	0.04

reduction degree balance (Table 3), for which one can estimate relative errors less than 3% and 2%, respectively. In particular, from the reduction degree balance it is evident that neither appreciable net accumulation nor consumption of $NADH_2^+$ was possible under anaerobic conditions, consistently with the necessary internal redox balance of anaerobic fermentation.

Table 1 also shows that, unlike oxygen supply, starting substrate level did not significantly influence molar production of ATP ($Y_{ATP/G}$) and CO_2 ($Y_{CO2/G}$). In fact, these parameters remained at the very low values characteristic of anaerobic fermentations (1.95–1.97 mol_{ATP} mol_G^{-1} and 1.47–1.59 mol_{CO2} mol_G^{-1}, respectively). As is well known, no net accumulation of ATP is possible in the cell under steady-state conditions, which means that the catabolic ATP production must necessarily be consumed by anabolic activities. Thus, the values of $Y_{ATP/G}$ and $Y_{X/G}$ listed in Table 1 have been used to estimate the average consumption of ATP for total synthesis of biomass precursors and their subsequent polymerization to biomass ($Y_{ATP/X}$=7.4 mol_{ATP} C-mol_{DW}^{-1}). This value is remarkably higher than that obtained by the authors (Converti and Perego, submitted) under semi-aerobic and aerobic conditions (4.2 mol_{ATP} C-mol_{DW}^{-1}) as well as that estimated from the data of Zeng et al. (1990c) in continuous culture (4.3 mol_{ATP} C-mol_{DW}^{-1}), thus confirming that the synthesis of bacterial biomass under anaerobic conditions consumes more energy than in the presence of oxygen.

As far as the yield of growth is concerned, either that referred to the consumption of glucose ($Y_{X/G}$) or that referred to the production of ATP ($Y_{X/ATP}$) were very low and scarcely influenced by S_o up to 54 g l^{-1}, while product inhibition of growth seemed to take place at higher S_o values. The average biomass yield on consumed glucose ($Y_{X/G}$=0.27 C-mol_{DW} mol_G^{-1}) was about half that obtained by Heijnen and Roels (1981) for the anaerobic growth of *Klebsiella aerogenes* (0.52–0.68 C-mol_{DW} mol_G^{-1}), likely due to the much lower maximum specific growth rates (μ_{max}=0.07–0.1 h^{-1}) compared to those reported by the above authors ($\mu_{max} \cong 0.5$ h^{-1}).

Finally, the values of $Y_{X/ATP}$ obtained in this work are much lower than those obtained for the anaerobic growth of *Aerobacter aerogenes* in chemostat (0.35–1.00 C-mol_{DW} mol_{ATP}^{-1}) and of many other microorganisms (0.48 C-mol_{DW} mol_{ATP}^{-1}) (Roels 1983). This result can be explained by the fact that during batch growth the environmental conditions progressively diverge from the optimum ones of continuous cultures. In other words, the progressive depletion of substrate and the accumulation of acidic products could make cell growth more expensive from the energetic point of view.

Appendix

Anaerobic metabolism of *Enterobacter aerogenes*

Butanediol production

$$\alpha\text{Glucose} + 2\alpha\text{NAD}^+ + 2\alpha\text{ADP} + 2\alpha\text{P}_i \rightarrow 2\alpha\text{Pyruvate} + 2\alpha\text{NADH}_2^+ + 2\alpha\text{ATP} + 2\alpha\text{H}_2\text{O} \quad (1)$$

$$2\alpha\text{Pyruvate} + \alpha\text{NADH}_2^+ \rightarrow \alpha 2,3\text{-Butanediol} + 2\alpha\text{CO}_2 + \alpha\text{NAD}^+ \quad (2)$$

Net (Eqs. 1 and 2):

$$\alpha\text{Glucose} + \alpha\text{NAD}^+ + 2\alpha\text{ADP} + 2\alpha\text{P}_i \rightarrow \alpha 2,3\text{-Butanediol} + \alpha\text{NADH}_2^+ + 2\alpha\text{ATP} + 2\alpha\text{CO}_2 + 2\alpha\text{H}_2\text{O} \quad (3)$$

Acetoin production

$$\beta\text{Glucose} + 2\beta\text{NAD}^+ + 2\beta\text{ADP} + 2\beta\text{P}_i \rightarrow 2\beta\text{Pyruvate} + 2\beta\text{NADH}_2^+ + 2\beta\text{ATP} + 2\beta\text{H}_2\text{O} \quad (4)$$

$$2\beta\text{Pyruvate} \rightarrow \beta\text{Acetoin} + 2\beta\text{CO}_2 \quad (5)$$

Net (Eqs. 4 and 5):

$$\beta\text{Glucose} + 2\beta\text{NAD}^+ + 2\beta\text{ADP} + 2\beta\text{P}_i \rightarrow \beta\text{Acetoin} + 2\beta\text{NADH}_2^+ + 2\beta\text{ATP} + 2\beta\text{H}_2\text{O} + 2\beta\text{CO}_2 \quad (6)$$

Pyruvate-formate lyase activity

$$\gamma\text{Glucose} + 2\gamma\text{NAD}^+ + 2\gamma\text{ADP} + 2\gamma\text{P}_i \rightarrow 2\gamma\text{Pyruvate} + 2\gamma\text{NADH}_2^+ + 2\gamma\text{ATP} + 2\gamma\text{H}_2\text{O} \quad (7)$$

$$2\gamma\text{Pyruvate} + 2\gamma\text{CoA} - \text{SH} \rightarrow 2\gamma\text{Acetyl} - \text{S} - \text{CoA} + 2\gamma\text{Formate} \quad (8)$$

Net (Eqs. 7 and 8):

$$\gamma\text{Glucose} + 2\gamma\text{NAD}^+ + 2\gamma\text{ADP} + 2\gamma\text{P}_i + 2\gamma\text{CoA} - \text{SH} \rightarrow 2\gamma\text{Formate} + 2\gamma\text{NADH}_2^+ + 2\gamma\text{ATP} + 2\gamma\text{H}_2\text{O} + 2\gamma\text{Acetyl} - \text{S} - \text{CoA} \quad (9)$$

c_1: Hydrogen production

$$\gamma_1\text{Formate} \rightarrow \gamma_1\text{CO}_2 + \gamma_1\text{H}_2 \quad (10)$$

c_2: Ethanol production

$$\gamma_2\text{Acetyl} - \text{S} - \text{CoA} + 2\gamma_2\text{NADH}_2^+ \rightarrow \gamma_2\text{Ethanol} + 2\gamma_2\text{NAD}^+ + \gamma_2\text{CoA} - \text{SH} \quad (11)$$

c_3: Acetate production

$$\gamma_3\text{Acetyl} - \text{S} - \text{CoA} + \gamma_3\text{P}_i \rightarrow \gamma_3\text{Acetylphosphate} + \gamma_3\text{CoA} - \text{SH} \quad (12)$$

$$\gamma_3\text{Acetylphosphate} + \gamma_3\text{ADP} \rightarrow \gamma_3\text{Acetate} + \gamma_3\text{ATP} \quad (13)$$

Net (Eqs. 12 and 13):

$$\gamma_3\text{Acetyl} - \text{S} - \text{CoA} + \gamma_3\text{ADP} + \gamma_3\text{P}_i \rightarrow \gamma_3\text{Acetate} + \gamma_3\text{CoA} - \text{SH} + \gamma_3\text{ATP} \quad (14)$$

Net (Eqs. 9–11 and 14):

$$\gamma\text{Glucose} + (2\gamma_2 - 2\gamma)\,\text{NADH}_2^+ + (2\gamma + \gamma_3)\,\text{ADP} + 2\gamma(2\gamma + \gamma_3)\,\text{P}_i \rightarrow (2\gamma - \gamma_1)\,\text{Formate} + \gamma_2\text{Ethanol} + \gamma_3\text{Acetate} + \gamma_1\text{H}_2 + 2\gamma\text{H}_2\text{O} + \gamma_1\text{CO}_2 + (2\gamma_2 - 2\gamma)\,\text{NAD}^+ + (2\gamma + \gamma_3)\,\text{ATP} \quad (15)$$

Lactate production

$$\delta\text{Glucose} + 2\delta\text{NAD}^+ + 2\delta\text{ADP} + 2\delta\text{P}_i \rightarrow 2\delta\text{Pyruvate} + 2\delta\text{NADH}_2^+ + 2\delta\text{ATP} + 2\delta\text{H}_2\text{O} \quad (16)$$

$$2\delta\text{Pyruvate} + 2\delta\text{NADH}_2^+ \rightarrow 2\delta\text{Lactate} + 2\delta\text{NAD}^+ \quad (17)$$

Net (Eqs. 16 and 17):

$$\delta\text{Glucose} + 2\delta\text{ADP} + 2\delta\text{P}_i \rightarrow 2\delta\text{Lactate} + 2\delta\text{ATP} + 2\delta\text{H}_2\text{O} \quad (18)$$

Succinate production

$$\varepsilon\text{Glucose} + 2\varepsilon\text{NAD}^+ + 2\varepsilon\text{P}_i \rightarrow 2\varepsilon\text{Phosphoenolpyruvate} + 2\varepsilon\text{NADH}_2^+ 2\varepsilon\text{H}_2\text{O} \quad (19)$$

$$2\varepsilon\text{Phosphoenolpyruvate} + 2\varepsilon\text{CO}_2 + 4\varepsilon\text{NADH}_2^+ \rightarrow 2\varepsilon\text{Succinate} + 2\varepsilon\text{P}_i + 4\varepsilon\text{NAD}^+ \quad (20)$$

Net (Eqs. 19 and 20)

$$\varepsilon\text{Glucose} + 2\varepsilon\text{NADH}_2^+ + 2\varepsilon\text{CO}_2 \rightarrow 2\varepsilon\text{Succinate} + 2\varepsilon\text{NAD}^+ + 2\varepsilon\text{H}_2\text{O} \quad (21)$$

Cell growth

$$\zeta\text{Glucose} + 1.38\zeta\text{NH}_3 \rightarrow 6\zeta\text{CH}_{1.74}\text{O}_{0.43}\text{N}_{0.23} + 1.32\zeta\text{O}_2 + 0.78\zeta\text{H}_2\text{O} \quad (22)$$

Anaerobic metabolism

Net (Eqs. 3, 6, 15, 18, 21, 22), where: $\alpha+\beta+\gamma+\delta+\varepsilon+\zeta=1$:

$$\text{Glucose} + [\alpha + 2\beta - 2(\gamma_2 - \gamma) - 2\varepsilon]\,\text{NAD}^+ + (2 + \gamma_3 - 2\varepsilon)\,\text{ADP} + (2 + \gamma_3 - 2\varepsilon)\,\text{P}_i + 1.38\zeta\text{NH}_3 \rightarrow \alpha\text{Butanediol} + \beta\text{Acetoin} + (2\gamma - \gamma_1)\,\text{Formate} + \gamma_2\text{Ethanol} + 2\delta\text{Lactate} + \gamma_3\text{Acetate} + 2\varepsilon\text{Succinate} + \gamma_1\text{H}_2 + [\alpha + 2\beta - 2(\gamma_2 - \gamma) - 2\varepsilon]\,\text{NADH}_2^+ + (2\alpha + 2\beta + \gamma_1 - 2\varepsilon)\,\text{CO}_2 + (2 + \gamma_3 - 2\varepsilon)\,\text{ATP} + (2 + 0.78\zeta)\,\text{H}_2\text{O} + 1.32\zeta\text{O}_2 + 6\zeta\text{CH}_{1.74}\text{O}_{0.43}\text{N}_{0.23} \quad (23)$$

References

De Mas C, Jansen NB, Tsao TG (1988) Production of optically active 2,3-butanediol by *Bacillus polymyxa*. Biotechnol Bioeng 31:366–377

Emerson RR, Flickinger MC, Tsao TG (1982) Kinetics of dehydration of aqueous 2,3-butanediol to methylethyl ketone. Industrial Eng Chem Prod Rev Dev 21:473–477

Garg SK, Jain A (1995) Fermentative production of 2,3-butanediol: a review. Biores Technol 51:103–109

Heijnen JJ, Roels JA (1981) Macroscopic model describing yield and maintenance relationship in aerobic fermentation processes. Biotechnol Bioeng 23:739–763

ISO (1997) Plastics – evaluation of the ultimate aerobic biodegradability in an aqueous medium – method by analysis of released carbon dioxide. In: International Organization for Standardization (ed) Drafts International Standards ISO/DIS 14852, ISO/TC 61/SC 5, pp 1–24

Jansen NB, Flickinger MC, Tsao TG (1984) Production of 2,3-butanediol from D-xylose by *Klebsiella oxytoca* ATCC 8724. Biotechnol Bioeng 26:362–369

Kosaric N, Lyng RP (1988) Microbial production of hydrogen. In: Rehm H-J, Reed G (eds) Biotechnology, vol 6B. Verlag Chemie, Weinheim, pp 101–134

Maddox IS (1988) Microbial production of 2,3-butanediol. In: Rehm H-J, Reed G (eds) Biotechnology, vol 6B. Verlag Chemie, Weinheim, pp 31–50

Magee RJ, Kosaric N (1987) The microbial production of 2,3-butanediol. Adv Appl Microbiol 32:89–161

Perego P, Fabiano B, Ponzano GP, Palazzi E (1998) Experimental study of hydrogen kinetics from agroindustrial by-products: optimal conditions for the production and fuel cell feeding. Bioproc Eng 19:205–211

Perego P, Converti A, Del Borghi A, Canepa P (2000) 2,3-Butanediol production by *Enterobacter aerogenes*: selection of the optimal conditions and application to food industry residues. Bioproc Eng 23:613–620

Ramachandran KB, Goma G (1987) Effect of oxygen supply and dilution rate on the production of 2,3-butanediol in continuous bioreactor by *Klebsiella pneumoniae*. Enzyme Microb Technol 9:107–111

Roels JA (1983) Energetics and kinetics in biotechnology. Elsevier, Amsterdam, pp 99–128

Sablayrolles JM, Goma G (1984) Butanediol production by *Aerobacter aerogenes* NRRL B199: effect of initial substrate concentration and aeration agitation. Biotechnol Bioeng 26:148–155

Tanisho S, Ishiwata Y (1994) Continuous hydrogen production from molasses by the bacterium *Enterobacter aerogenes*. Int J Hydrogen Energy 19:807–812

Tanisho S, Ishiwata Y (1995) Continuous hydrogen production from molasses by fermentation using urethane foam as a support of flocks. Int J Hydrogen Energy 20:541–545

Tanisho S, Kamiya N, Wakao N (1989) Hydrogen evolution of *Enterobacter aerogenes* depending on culture pH: mechanism of hydrogen evolution from NADH by means of membrane-bound hydrogenase. Biochim Biophys Acta 973:1–6

Tanisho S, Kuromoto M, Kadokura N (1998) Effect of CO_2 removal on hydrogen production by fermentation. Int J Hydrogen Energy 23:559–563

Voloch M, Jansen NB, Ladish MR, Tsao GT, Narayan R, Rodwell VW (1985) 2,3-Butanediol. In: Blanch HW, Drew S, Wang DIC (eds) Comprehensive biotechnology; the principles, applications and regulations of biotechnology in industry, agriculture and medicine. Pergamon, Oxford, pp 933–944

Zajic JE, Kosaric N, Brosseau JD (1987) Microbial production of hydrogen. Adv Biochem Eng 9:57–109

Zeng A-P, Deckwer W-D (1991) A model for multiproduct-inhibited growth of *Enterobacter aerogenes* in 2,3-butanediol fermentation. Appl Microbiol Biotechnol 35:1–3

Zeng A-P, Deckwer W-D (1992) Utilization of the tricarboxylic acid cycle, a reactor design criterion for the microaerobic production of 2,3-butanediol. Biotechnol Bioeng 40:1078–1084

Zeng A-P, Biebl H, Deckwer W-D (1990a) 2,3-Butanediol production by *Enterobacter aerogenes* in continuous culture: role of oxygen supply. Appl Microbiol Biotechnol 33:264–268

Zeng A-P, Biebl H, Deckwer W-D (1990b) Effect of pH and acetic acid on growth and 2,3-butanediol production of *Enterobacter aerogenes* in continuous culture. Appl Microbiol Biotechnol 33:485–489

Zeng A-P, Ross A, Deckwer W-D (1990c) A method to estimate the efficiency of oxidative phosphorilation and biomass yield from ATP of a facultative anaerobe in continuous culture. Biotechnol Bioeng 36:965–969

Zeng A-P, Byun T-G, Posten C, Deckwer W-D (1994) Use of respiratory quotient as a control parameter for optimum oxygen supply and scale-up of 2,3-butanediol production under microaerobic conditions. Biotechnol Bioeng 44:1107–1114