Butanol Production by a Butanol-Tolerant Strain of *Clostridium acetobutylicum* in Extruded Corn Broth

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By employing serial enrichment, a derivative of *Clostridium acetobutylicum* ATCC 824 was obtained which grew at concentrations of butanol that prevented growth of the wild-type strain. The parent strain demonstrated a negative growth rate at 15 g of butanol/liter, whereas the SA-1 mutant was still able to grow at a rate which was 66% of the uninhibited control. SA-1 produced consistently higher concentrations of butanol (from 5 to 14%) and lower concentrations of acetone (12.5 to 40%) than the wild-type strain in 4 to 20% extruded corn broth (ECB). Although the highest concentration of butanol was produced by SA-1 and the wild-type strain in 14% ECB, the best solvent ratio with respect to optimizing butanol and decreasing acetone occurred between 4 and 8% ECB for SA-1. SA-1 demonstrated higher conversion efficiency to butanol than the wild-type strain at every concentration of ECB tested. Characterization of the wild-type and SA-1 strain in 6% ECB demonstrated the superiority of the latter in terms of growth rate, time of onset of butanol production, carbohydrate utilization, pH resistance, and final butanol concentration in the fermentation broth.

Diminishing natural oil and gas resources have made the search for alternative fuel sources increasingly important. Butanol, in addition to its many uses as a chemical feedstock, is among the alternatives. The acetone-butanol-ethanol fermentation with *Clostridium acetobutylicum* has a history of using primarily molasses or corn as carbohydrate source. Physiological determinations associated with solvent production have been carried out with corn mash (19) and, more recently, molasses (3, 15). In the midwest, interest in butanol production from corn has been reinitiated due to the periodic abundance of mycotoxin-contaminated waste corn and a shortage of energy on the farm. A process of continuous extrusion cooking of whole kernel corn as been developed recently. Advantages over conventional cooking methods may include increased alcohol yields, energy reduction, and by-product improvement (6). Nothing is known about the physiological changes associated with solvent production with extruded corn.

End-product inhibition has long been recognized as a barrier to high productivities in many industrial fermentations. Butanol toxicity, of particular importance, appears to be the primary limitation in the typical butanol fermentation process utilizing *C. acetobutylicum* (17, 21). A butanol concentration of 13 g/liter in the fermentation broth is inhibitory (21, 22). An increase in the concentration of butanol in the fermentation broth should facilitate recovery, thereby reduc-

ing distillation costs. Recently, a relatively simple enrichment procedure was developed for deriving an ethanol-tolerant mutant from *Clostridium thermocellum* (10). The objective of this study was to develop a butanol-tolerant mutant from *C. acetobutylicum* and subsequently characterize this strain in a batch fermentation of extruded corn to determine whether a more efficient butanol fermentation was established.

MATERIALS AND METHODS

Strains and culture maintenance. *C. acetobutylicum* ATCC 824, 4259, 10132, NRRL B-591, and *Clostridium saccharoperbutylacetonicum* ATCC 27022 were grown anaerobically at 37°C for 24 h and maintained at −196°C in 4.2% corn starch soluble medium (CSSM) containing (in grams per liter): corn starch (Sargent-Welch Co., Skokie, Ill.), 42; KH_2PO_4, 0.75; K_2HPO_4, 0.75; $MgSO_4$, 0.02; $MnSO_4 \cdot H_2O$, 0.01; $FeSO_4 \cdot 7H_2O$, 0.01; NaCl, 1.0; cysteine, 0.05; yeast extract, 10; asparagine $\cdot H_2O$, 2.0; and $(NH_4)_2SO_4$, 2.0. This is a modification of the soluble medium described by Moreira et al. (17) with corn starch as the carbohydrate source. Similarly, the butanol-tolerant SA-1 strain was cultivated and maintained in CSSM containing 18 g of *n*-butanol/liter (Mallinckrodt, Inc., St. Louis, Mo.). Cultures were transferred into fresh fluid thioglycolate medium (Difco Laboratories, Detroit, Mich.) at bimonthly intervals, incubated for 24 h at 37°C, and subsequently inoculated into fresh CSSM with or without added butanol.

Culture conditions. Experimental studies were routinely carried out under anaerobic atmosphere (85% N_2, 10% CO_2, and 5% H_2) in a Coy anaerobic chamber

(Coy Laboratory Products, Inc., Ann Arbor, Mich.) at 37°C. Medium, distilled water, and *n*-butanol were placed in the anaerobic chamber at least 24 h before use to ensure removal of dissolved oxygen. CSSM was used as the inoculum medium for studies involving 6% extruded corn broth (ECB). Glucose soluble medium (GSM) and soluble starch medium (SSM) were used for adaptation and challenge studies, respectively. The corn starch in CSSM was replaced by glucose (47 g/liter) in GSM and by soluble starch (20 g/liter; J. T. Baker Chemical Co., Phillipsburg, N.J.) in SSM. Growth in CSSM and ECB was evaluated by plate counts on tryptic glucose yeast extract medium (13). Extruded corn feedstock was prepared by extrusion through a Brady 206 Crop Cooker (Koehring Inc., Appleton, Wis.) operated at 166°C in the presence of 2% (vol/wt) water. ECB was prepared by suspending extruded corn in distilled water and autoclaving at 121°C for 15 min.

Strain selection. Stock cultures of the five wild-type strains were grown to late-logarithmic phase in CSSM and 5% (vol/vol) inoculated into 6% (wt/vol) ECB. A gas chromatographic assay of butanol concentration in ECB was carried out at 24-h intervals up to 96 h to determine the most suitable strain for subsequent adaptation and challenge studies.

Development of butanol tolerance. The butanol-tolerant strain was derived from *C. acetobutylicum* ATCC 824 by a serial enrichment procedure similar to the process used to develop ethanol tolerance in *C. thermocellum* (10) and *C. thermosaccharolyticum* (23). Portions (1 ml) of late-exponential-phase cultures of the parent strain were inoculated into Coleman cuvettes (14 by 105 mm; Coleman Instruments, Inc., Maywood, Ill.) containing 8 ml of pretempered GSM at 1.25 times the normal concentration. After 30 min of incubation, the cultures were challenged with 1 ml of *n*-butanol solution suitably diluted with sterile distilled water to a final concentration in GSM of 5 g/liter. Growth was monitored by measuring the optical density at 585 nm (OD_{585}), using a Coleman model 6A spectrophotometer. Cultures with an OD_{585} of 0.8 or the highest OD_{585} attainable, were transferred sequentially to fresh media containing increasing concentrations of *n*-butanol. Butanol concentrations in GSM were based on the quantities added to the cuvettes and present in the inoculum and disregarded the quantity produced during the experimental time interval. After 12 transfers, a strain capable of growth in the presence of high concentrations of *n*-butanol was obtained.

Challenge studies. The growth response of *C. acetobutylicum* 824 and the tolerant derivative was evaluated in the presence of butanol challenges (0 to 20 g/liter). The parent and mutant strains were grown in SSM to an OD_{585} of 0.8, and 0.5 ml was transferred to Coleman cuvettes containing 9 ml of SSM at 1.11 times its normal concentration. After incubating for 30 min, the cultures were challenged with 0.5 ml of a serially diluted *n*-butanol solution. Final butanol concentrations were based on the amount of butanol added. The butanol carry-over from the SA-1 inoculum culture tube was reduced by 3× transfer in SSM without added butanol before conducting the experiments. The growth response was recorded by following OD_{585} as a function of time (data not shown). The time of butanol addition was designated time zero. Maximum specific growth rates (μ) were calculated from the slopes of the

least-squares regression lines, which fitted the natural logarithm of OD_{585} as a function of time. Regression analysis was performed on values taken from the linear portion of the growth-response curve plotted on semilogarithmic paper.

Characterization of fermentation profiles. To maximize solvent formation with particular emphasis on solvent ratios (butanol:acetone:ethanol) as well as final butanol concentration, we examined the effect of various concentrations of extruded corn on the solvent production capacity by *C. acetobutylicum* 824 and SA-1 after 96 h of fermentation. Both strains were inoculated at the 5% (vol/vol) level into 4 to 20% (wt/vol) ECB. Solvent ratios were calculated at each concentration of ECB based on the quantity of total solvent produced. Conversion efficiency of extruded corn to butanol was based on grams of starch added as extruded corn in the fermentation broth.

C. acetobutylicum strains 824 and SA-1 were evaluated for growth rate, pH profile, extracellular α-amylase activity, total carbohydrate utilization, and butanol concentration and yield in 6% ECB. A late-exponential-phase CSSM culture was diluted 1:100 in 0.1% peptone to 10^6 cells per ml and used at the 5% (vol/vol) level to inoculate 400 ml of ECB in a 1-liter screw-top Erlenmeyer flask. Quantitation of total carbohydrate was made instead of starch utilization due to a recent report on the production of carboxymethyl cellulase and cellobiase by a strain of *C. acetobutylicum* (1). Celluloses and hemicelluloses account for 10% of the total carbohydrates in corn (11).

Analytical methods. A Varian 3700 gas chromatograph (Varian Inc., Sunnyvale, Calif.) equipped with flame ionization detector was used for solvent assays. Portions of 0.5-ml samples were injected into a stainless steel column (6 ft by ⅛ in. [1.8 m by 3.2 mm]) packed with 5% Carbowax 20 M TPA on Chromosorb WHP 80/100 mesh and operated at 50 to 100°C programmed at 8°C/min. Temperatures of the inlet and detector were held at 100 and 250°C, respectively. Gas flow rates were: N_2 (carrier gas), 20 ml/min; and H_2, 30 ml/min; air, 300 ml/min. An *n*-propanol internal standard was used. The retention times for acetone, ethanol, *n*-propanol, and butanol were 0.8, 1.56, 2.34, and 3.75 min, respectively. Analysis and interpretation of data was accomplished with a Hewlett-Packard 3390A Integrator (Hewlett-Packard Inc., Avondale, Pa.).

Residual total carbohydrate was measured by using the colorimetric procedure described by Neish (18). Quantitative determination of extracellular α-amylase activity was based on the amount of reducing sugars liberated during the enzymatic hydrolysis of starch (7). Biochemical characterization of strains was accomplished with the API-20A Anaerobic Diagnostic System (Analytab Products, Plainview, N.Y.).

RESULTS

Initial strain selection. Four strains of *C. acetobutylicum* and one strain of *C. saccharoperbutylacetonicum* were compared for the ability to produce butanol in 6% ECB. *C. acetobutylicum* ATCC 824 produced significantly more butanol (7.9 g/liter) than the other strains after a 96-h fermentation period. *C. acetobutylicum* NRRL B591 produced 2.6 g of butanol/liter, whereas
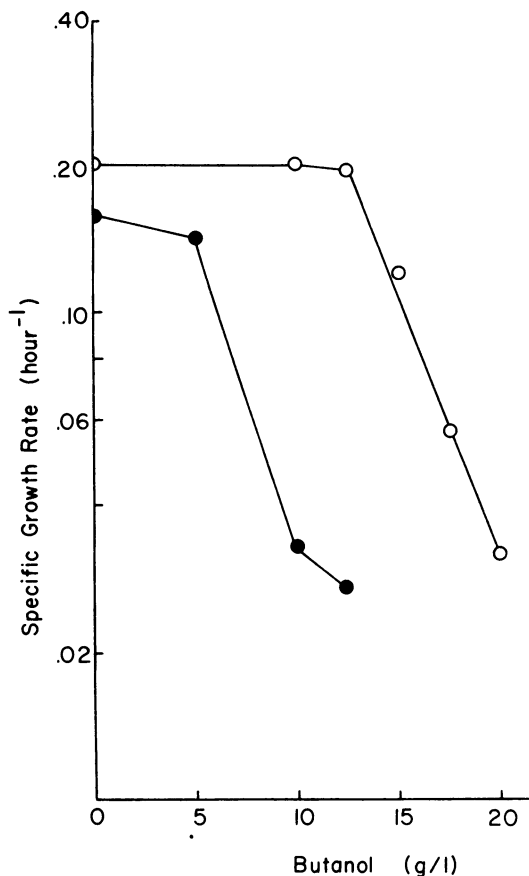
FIG. 1. Maximum specific growth rate (μ) of *C. acetobutylicum* ATCC 824 (●) and SA-1 (○) as a function of butanol concentration.
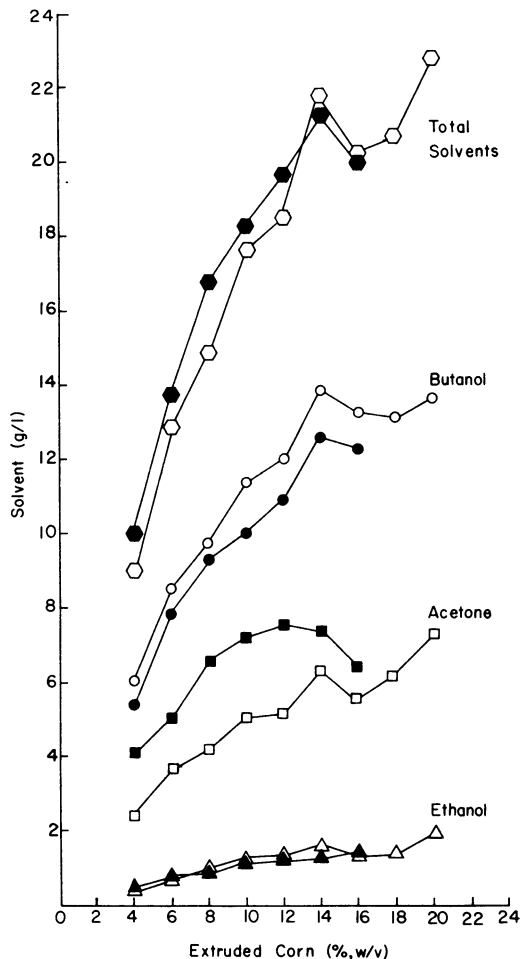

FIG. 2. Effect of various concentrations of extruded corn on solvent formation by *C. acetobutylicum* ATCC 824 (closed symbols) and SA-1 (open symbols).

the other strains produced this solvent at concentrations less than 2.0 g/liter. *C. acetobutylicum* 824 was selected as the strain for further studies.

Development of a butanol-tolerant mutant. The butanol-tolerant mutant, designated SA-1, was derived from the parent strain by the stepwise enrichment procedure described above. After a growth arrest of 144 h, the SA-1 mutant was able to grow to an OD_{585} value of 0.54 in GSM containing 18.6 g of butanol/liter. SA-1 demonstrated similar Gram stain reaction and microscopic morphology as the parent strain, although this strain had lost the ability to ferment esculin and acquired raffinose, sorbitol, and melezitose fermenting ability. The butanol tolerance of SA-1 was further indicated by its ability to grow stably and remain viable for long periods in both GSM and CSSM containing butanol selection pressure.

Response of *C. acetobutylicum* to butanol challenge. A semilogarithmic plot of μ versus butanol concentration for *C. acetobutylicum* 824 and SA-1 is shown in Fig. 1. The growth response of SA-1 indicates higher tolerance to butanol than the wild-type strain at all challenge concentrations. The specific growth rate of the parent strain was inhibited 50% by 7 g of butanol/liter, whereas SA-1 was inhibited 50% by 15.5 g of butanol/liter. At a butanol concentration of 15 g/liter, the parent strain had a negative growth rate (possibly due to cellular lysis), whereas the SA-1 mutant was able to grow at a rate which was 66% of the uninhibited control. The specific growth rate of SA-1 remained positive even at the 20 g of butanol/liter challenge. Also, SA-1 demonstrated a faster growth rate ($\mu = 0.197$) for the unchallenged control than the wild-type strain ($\mu = 0.156$) in 2% SSM. The growth rate of SA-1 in glucose-based GSM was even faster

(μ = 0.235) than that in SSM starch-containing medium.

Effect of extruded corn concentration on solvent formation. The effect of various concentrations of extruded corn (4 to 20% [wt/vol]) on solvent formation by *C. acetobutylicum* 824 and SA-1 after 96 h of fermentation can be seen in Fig. 2. As the concentration of ECB was increased to 14%, so too was the solvent produced by both strains. The highest concentration of butanol was produced in 14% ECB, with SA-1 producing 13.9 g/liter and 824 producing 12.6 g/liter. The SA-1 mutant consistently produced higher levels of butanol (from 5 to 14%) and consistently less acetone (from 12.5 to 40%) than the wild-type strain between 4 and 16% ECB. Since the reduction in acetone by SA-1 was greater than the gain in butanol, total solvent concentration was lower for SA-1 than the parent strain at all concentrations of ECB except 14 and 16%, where there was a slight decrease in ability of the wild-type strain to produce acetone.

The effect of various concentrations of ECB on the butanol:acetone:ethanol solvent ratio by the parent strain and SA-1 can be seen in Table 1. Although the highest concentration of butanol occurred at 14% ECB, optimal solvent ratios with respect to optimizing butanol and decreasing acetone occurred between 4 and 8% ECB for SA-1. Consistent with data from Fig. 2, SA-1 produced a higher fraction of solvent as butanol and a lower fraction of acetone at all concentrations of ECB than the parent strain. For *C. acetobutylicum* 824, as the concentration of

TABLE 1. Effect of various concentrations of ECB on the solvent ratio and conversion efficiency to butanol by *C. acetobutylicum* ATCC 824 and SA-1[a]

% (wt/vol) Extruded corn	Solvent ratio (butanol:acetone: ethanol)		Conversion efficiency[b]	
	824	SA-1	824	SA-1
4	54:40:6	68:27:5	0.191	0.213
6	57:37:6	66:28:6	0.185	0.199
8	55:39:6	66:28:6	0.162	0.170
10	55:39:6	65:29:6	0.140	0.160
12	58:38:6	65:28:7	0.127	0.140
14	59:35:6	64:29:7	0.126	0.139
16	61:32:7	65:28:7	0.107	0.115
18	ND[c]	64:29:7	ND	0.101
20	ND	60:32:8	ND	0.095

[a] Determined after 96 h at 37°C.

[b] Determined by dividing the grams of butanol produced by the grams of starch added as extruded corn to the fermentation broth. This is based on whole kernel corn containing 71.5% starch on a dry weight basis (16).

[c] ND, No data.

ECB increased, the fraction of butanol increased and acetone decreased, whereas the reverse was true for strain SA-1. Also, for SA-1 the fraction of ethanol increased as the concentration of ECB was increased to 20%. The conversion efficiency to butanol decreased dramatically with increasing concentrations of ECB for both SA-1 and the wild-type strain (Table 1). SA-1, however, demonstrated higher conversion efficiency than the wild-type strain at every concentration of ECB tested. A 6% level was chosen as the concentration of ECB to be used in further studies.

Physiological characterization. Cell growth, pH, residual total carbohydrate, and butanol concentrations were monitored during a small-scale batch fermentation in 6% ECB to characterize and compare the SA-1 mutant with the parent strain (see Fig. 3). Cell growth of the wild-type strain was arrested for 6 h, whereas growth of SA-1 was exceptionally rapid with almost no lag period. The generation time of SA-1 was significantly shorter (32.8 min) than the wild-type strain (57.9 min). Consequently, the time for the parent strain to reach stationary phase was 30 h, compared to only 12 h for SA-1. A similar growth response was obtained for SA-1 and the wild-type strain in 4.2% CSSM (data not shown).

During the first 30 h, the pH of ECB inoculated with the parent strain fell rapidly from 5.8 to 4.0 in response to exponential growth. Below pH 3.8 the cell concentration began to decrease rapidly. The pH continued to drop to the lowest value (3.42, the pH breakpoint) at which point the rate of decrease in cell concentration slowed markedly and the production of butanol was initiated. An increase in pH, caused by the conversion of acid to solvent (5), proceeded until a stabilized pH range of 3.8 to 3.9 was reached after 96 h. The greatest increase in butanol concentration took place between 48 and 96 h, which coincided with the pH readjustment, stabilization of cell concentration, and a marked reduction in residual total carbohydrate. Total carbohydrate, present at a concentration of 40.5 g/liter at the start of the 6% ECB fermentation, was reduced to 6.8 g/liter after 144 h. This represents 83.2% utilization of total carbohydrate by the parent strain during this fermentation.

The maximum cell number for SA-1 in 6% ECB was 60% higher, and the pH drop was steeper, than that for the wild-type culture. The lowest pH of 3.47 at 38 h coincided with initiation of butanol production and stabilization of cell concentration. SA-1 was more resistant to lower pH than the parent strain since the decline in stationary-phase cell number between pH 3.8 and 3.4 was 1.25 × log for the wild-type strain
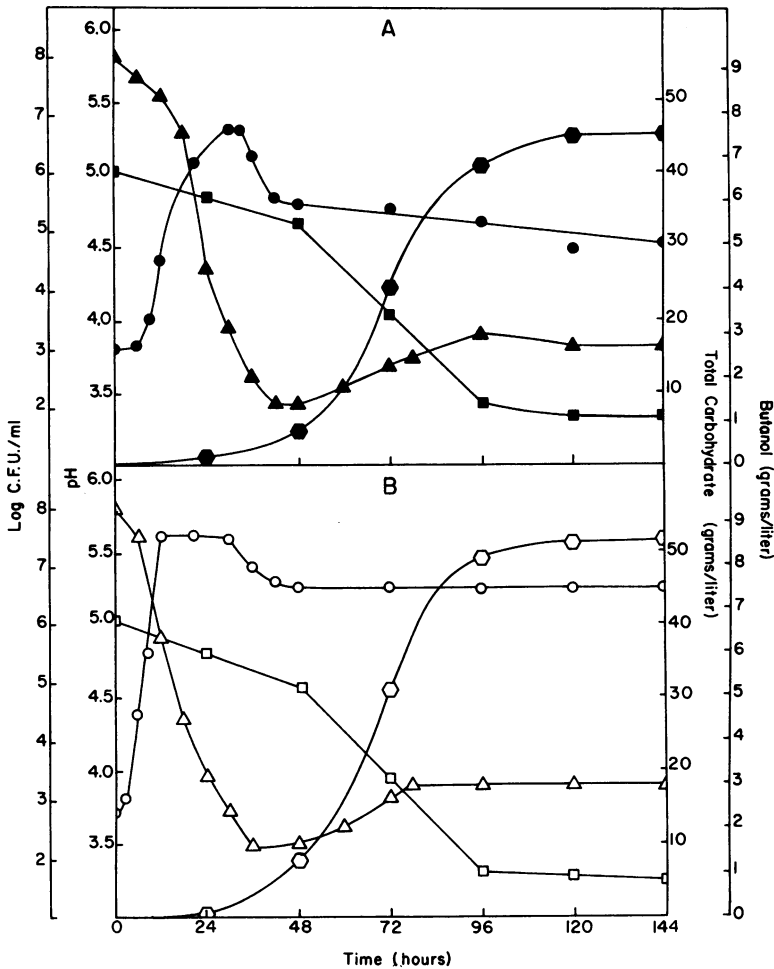
FIG. 3. Physiological characterization of *C. acetobutylicum* (A) ATCC 824 and (B) SA-1 during small-scale batch fermentation in 6% ECB. Symbols: ● and ○, cell growth (log CFU/ml); ▲ and △, pH; ■ and □, total carbohydrate (g/liter); ◐ and ◓ , butanol (g/liter).

and only 0.875 × log for SA-1. The final butanol concentration for SA-1 was 8.6 g/liter, 13.2% higher than that produced by the parent strain (7.6 g/liter). In addition, butanol was produced 9 to 12 h earlier by SA-1 than 824, possibly because of earlier attainment of the pH break-point (38 h for SA-1 and 46 h for 824) (2). The residual total carbohydrate for SA-1 was 4.8 g/liter after 144 h and represents 88.1% utilization, a slight improvement over the performance of the wild-type strain.

The ECB fermentation profile seen in Fig. 3 is a consequence of using a very low inoculum level (i.e., 0.05%). When we employed a 5% inoculum of 824, rapid butanol production occurred between 24 and 48 h, and more than 90% of the final butanol concentration was produced

by 66 h. SA-1 produced butanol rapidly between 12 and 48 h, and more than 95% of the final butanol concentration was produced by 60 h (data not shown).

Table 2 shows a comparison of three strains of *C. acetobutylicum* with respect to growth rate, carbohydrate utilization, butanol concentration, and yield in 6% ECB. α-Amylase activity was determined on stationary-phase cultures grown in CSSM. The percentage of total corn carbohydrate utilized increased from 64.2% for strain NRRL B-591 to 83.2% for strain 824 and further to 88.1% for mutant SA-1. These values correlate with an increase in extracellular α-amylase activity for these cultures, as well as correspondingly higher final concentrations of butanol in 6% ECB. The butanol yields (grams

TABLE 2. Comparison of *C. acetobutylicum* NRRL B591, ATCC 824, and SA-1 with respect to generation time, carbohydrate utilization, butanol concentration, butanol yield, and extracellular α-amylase activity

Strain	Gener-ation time (min)	Carbohy-drate uti-lization (%)[a]	Buta-nol con-centra-tion (g/liter)[a]	Butanol yield[b]	α-Amylase activity (U/ml)[c]
NRRL B591		64.2	2.7	0.10	0.73
ATCC 824	57.9	83.2	7.6	0.23	1.07
SA-1	32.8	88.1	8.6	0.24	1.14

[a] These values were determined after 96 h of fermentation at 37°C in 6% ECB.

[b] Determined by dividing the grams of butanol produced by the grams of total carbohydrate utilized.

[c] Activity (1 U) was defined as the amount of enzyme releasing 1 μmol of reducing sugar per min at 37°C.

of butanol produced divided by the grams of total carbohydrate utilized) were approximately the same for 824 (0.23) and SA-1 (0.24) in spite of the 13.2% increase in final butanol concentration by SA-1 over the wild-type strain in 6% ECB.

DISCUSSION

The relationship between microbial alcohol tolerance and final concentration in the fermentation broth has been recognized for some time (20–22). The SA-1 mutant demonstrated a 121% increase in butanol tolerance and a further 13.2% improvement in the ability to produce butanol over the wild-type strain in 6% ECB. The limitation to higher production by the tolerant strain may lie with more efficient utilization of starch (see below). Although there is one report (14) on amplifying the tolerance of *C. acetobutylicum* to butanol by using continuous cultivation, this is the first report on successfully adapting this microorganism to higher butanol tolerance (above 13 g/liter) by using serial transfer in batch culture.

Based on a 30% conversion of carbohydrates to solvents, the 1.3% butanol tolerance of commercially useful strains (21) fixes the initial concentration of carbohydrate in the broth between 6 and 8%. If the carbohydrate in the medium is raised above 6%, the concentration of solvent increases, although the fermentation may not be as efficient (20). Since we had demonstrated that SA-1 was significantly more tolerant to butanol, we were interested in the effects of increasing the corn concentration on solvent formation and the conversion efficiency to butanol. Although the highest concentration

of butanol produced by both SA-1 and the parent strain occurred in 14% ECB, the efficiency of conversion of extruded corn (based on starch added) to total solvent decreased dramatically from 0.35 at 4% to 0.18 at 16% ECB for both wild-type and SA-1. The conversion efficiency to butanol also decreased over the range of corn tested, although the conversion efficiency was higher for SA-1 than the wild-type strain at each concentration of corn tested (Table 1).

To compare the whole kernel extruded corn fermentation with the industrial process using partially refined corn meal, conversion efficiencies were normalized by basing them on starch added. This is important, since the proportion of starch increases from 71.5% in whole kernel corn to 85.5% in refined corn meal at the expense of reduction in corn oil and fiber (16). Industrially, *C. acetobutylicum* is able to produce about 26.5% solvents based on 6.5% degerminated corn meal, with a solvent ratio of 60% butanol, 30% acetone, and 10% ethanol (22). The corresponding conversion efficiency based on a starch content of 5.6% can be calculated to be 0.186. This conversion efficiency compares favorably with that obtained for strain 824 (0.185; Table 1) in 6% extruded corn when based on 4.3% starch added. Therefore, the conversion efficiency for 824, based on starch added, is equivalent to that achieved industrially. The butanol-tolerant mutant SA-1 is significantly improved in this regard.

Since the acetone-butanol-ethanol fermentation would have greatest value if butanol were optimized, many attempts have been made to find ways to reduce acetone and increase the yield of butanol. Limited success in increasing the proportion of butanol at the expense of acetone has been achieved by strain selection (20), chemical addition (12), and variation in type and amount of carbohydrate (4). Ryden (21) reported no change in the solvent ratio after use of an adaptation procedure on *C. acetobutylicum*. The optimal solvent ratio for SA-1 with respect to butanol optimalization occurred at low concentrations of added ECB (Table 1), whereas high concentrations of ECB were needed to maximize the butanol fraction when the parent strain was employed. Although the total solvent concentration for SA-1 was lower than 824 at all but the highest concentrations of ECB, the SA-1 mutant produced consistently more butanol and less acetone at every concentration of ECB (Table 1, Fig. 2). These results suggest a greater efficiency of conversion to butanol, as well as an end-product shift in the metabolic pathway of SA-1, with greater amounts of butanol being produced at the expense of acetone.

The metabolism of *C. acetobutylicum* is such that acids are produced first (5) followed by a

shift in the fermentation to acetone and butanol. Although the mechanism is still under debate, the timing and magnitude of this shift appear to depend on the pH and composition of the growth medium as well as on the relative concentration of acetate and butyrate (2, 8, 9).

Comparison of the wild-type strain with the SA-1 mutant under actual batch fermentation conditions indicated that SA-1 was better suited to growth and butanol production in 6% ECB than the wild-type strain. This is consistent with the idea that the butanol adaptation process selected for a faster-growing microorganism. The faster growth rate and higher final stationary-phase cell population of SA-1, together with more rapid attainment of the pH breakpoint, resulted in a greater amount of butanol being produced much earlier in the fermentation. Recently it was demonstrated (8) that the mere attainment of a low pH value in the fermentation does not of itself provoke solvent synthesis. Rather, it was concluded that low pH is a prerequisite for solvent production, with relatively high concentrations of acetate and butyrate having an additional and more specific "triggering" effect on solvent production.

The more rapid attainment of the pH breakpoint by SA-1 suggests that the acids butyrate and acetate are produced sooner in the fermentation. The earlier onset of acid formation may be a major factor in the improved production of the secondary metabolite butanol during the idiophase, since Gottschal and Morris (8) indicate high concentrations of acetate and butyrate increase the final solvent yields. The faster onset of butanol formation by SA-1 over the wild-type strain has important economic implications for this fermentation.

The 13.2% increase in butanol concentration by SA-1 over 824 in 6% ECB appears to be the result of improved butanol tolerance as well as improved growth characteristics for this strain. Also, greater α-amylase activity of SA-1 versus wild-type 824 or B-591 was found to correlate with higher carbohydrate utilization, higher stationary cell population, and higher levels of butanol in the final fermentation broth. The improved growth response of SA-1 versus 824 in starch medium, data indicating SA-1 had a higher growth rate in glucose than starch-based medium, and the decline in conversion efficiency with increasing concentrations of ECB are consistent with the need for further enhancement of amylase activity in the butanol-tolerant SA-1 strain.

Strain SA-1 is clearly superior to the wild-type strain in terms of growth rate, butanol tolerance, onset time for butanol production and final concentration, pH resistance, carbohydrate utilization, conversion efficiency to butanol, and sol-vent ratio. Higher α-amylase activity in the SA-1 strain may be responsible for the more efficient utilization of corn carbohydrate (90% is corn starch) and a greater concentration of butanol being produced. Further amplification of amylase enzymatic activity in the butanol-tolerant SA-1 strain may increase the final butanol concentration, as well as the conversion efficiency at high concentrations of added ECB.

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