0273-2289/90/2425—0545$02.00

Influence of Feedstock Source on Biocatalyst Stability and Behavior, and on Reactor Performance, in Continuous Intensified Ethanol Fermentation

H. F. DE CASTRO AND J. D. BU'LOCK*

Weizmann Microbial Chemistry Laboratory, University of Manchester, Manchester M13 9PL, UK

ABSTRACT

An ethanol process based on a gas-lift tower fermenter arrangement was used as a model system to show the strong dependence of reactor behavior on the developing chemical environment within the reactor. The reactor performance limits for realistic substrates—starch and molasses—are characterized and compared with those attainable on an ideal substrate, glucose.

Index Entries: Biocatalyst; stability; feedstock; ethanol fermentations; reactor performance.

INTRODUCTION

We have developed and described an intensified continuous ethanol fermentation process using a strongly flocculent yeast strain (*1,2*). In principle, a similar system can be used for biotransformations that use immobilized particles with similar pellet or floc morphology (*3*). The simplicity of design and construction, coupled with high versatility and productivity (*4,5*), make this configuration suitable for scale-up; however, the characteristics of the fermentation will depend on the quality of the feedstock. Our main aim was to verify this potential application, and the limits of the reactor performance, using substrates of greater complexity than the synthetic glucose-based media used in developing the reactor

*Author to whom all correspondence and reprint requests should be addressed.

configuration. This in turn relates to the chemical and physical characteristics of the yeast strain in a given environment.

In global terms, practical production of ethanol uses either sucrose or starch as a feedstock. Each involves established commercial processes (*6,7*), but more developed systems are needed to improve process economics. Specific problems arise with these practical feedstocks. Starch substrates require a hydrolytic step, either as pretreatment or integrated with fermentation. Sucrose is most cheaply available as molasses, containing a high proportion of salts and other nonfermentables.

For this reason, an experimental protocol was established for each substrate. For a substrate based on blackstrap molasses, the active self-immobilizing yeast floc, as originally applied in glucose fermentations, was used. Alternatively, the yeast flocs were used in cosuspension with an immobilized glucoamylase preparation, selected because its rheological properties are similar to, and therefore compatible with, the yeast particles, and the mixed biocatalyst was applied to the simultaneous saccharification and fermentation (SSF) of a dextrinized starch substrate. Here we show that (a) with the molasses system, it is the nonfermentable components of the feedstock that impose new limits on reactor performance, which remains satisfactory below those limits; (b) with the SSF system, the tendency of the growing yeast to dilute the glucoamylase makes the system intrinsically unstable.

MATERIALS AND METHODS

Feedstocks

Glucose was commercial grade dextrose monohydrate (Cerelose) from CPC Ltd. (Trafford Park, Manchester). Sucrose was supplied as blackstrap molasses, a gift of Tate and Lyle Ltd. (UK). Dextrinized starch was a soluble powder, trade name Snowflake, produced by enzymatic conversion of maize starch by CPC Ltd. (UK). Other materials were reagent grade.

Analytical Methods

Methods for sugar, biomass, and ethanol analyses were as previously described (*4,8*). Glucoamylase activity of catalysts was measured by the method of Maeda et al. (*9*). Fermentation of both feedstocks was also monitored by high-performance liquid chromatography (HPLC) to follow residual sugars composition (*10*).

Biocatalyst Preparations

The yeast biocatalyst, a high flocculent strain of *Saccharomyces uvarum* (strain 17 from this laboratory) that forms a well-suspended particulate

Table 1
Characteristics of Immobilized Glucoamylase Catalyst

Carrier type	Gel beads activated with glutaraldehyde
Immobilization method	Passive adsorption
Yield of bound activity	30%
Activity of bound enzyme	800–1400 μmol glucose/g.min
Density when swollen	1.063
Mean diameter when swollen	500 μm
Maximum dextrin conversion	94%
Storage stability (4°C, 90 d)	Little apparent loss
Operating stability	4% reduction after 5 cycles
Effect of ionic strength	100% soluble in 0.5*M* acetate buffer, pH 5.0
Glucose generation rate from dextrin	8.6 g glucose (g dry wt catalyst)$^{-1}$h^{-1}

dispersion, was grown in the reactor. A loopful of cells was transferred from agar slants to 500-mL shake flasks containing 100 mL of sterilized MYGP broth containing 0.3% malt extract, 0.3% yeast extract, 0.5% bactopeptone, and 11% glucose hydrate at pH 5.5. The flasks were shaken (120 rpm, 2.5-cm throw) for 24 h at 30°C. The resulting suspension was used as a 10% (v/v) inoculum for the reactor. Yeast particles thus prepared transform glucose into ethanol at a specific rate of 0.23 g glucose (g dry weight)$^{-1}$h^{-1}.

For the glucoamylase catalyst, the support carrier was a cross-linked acrylate gel in bead form, a gift of Allied Colloids Ltd (Cleckheaton, W. Yorks, UK) (Development Product 7-2896). The dry beads were stirred for 1 h in distilled water (100 mL/1g beads); then the swollen beads were soaked for 1 h in a glutaraldehyde solution (5% v/v in 0.02*M* acetate buffer, pH 4.5) and poured into a 40-mm mesh metal sieve. The treated beads were thoroughly washed with 0.02*M* acetate buffer, pH 4.5, and 0.3*M* KCl solution alternately. For each 1 g of dry beads, 200 mL of glucoamylase solution (100 mL of manufacturer's preparation with 100 mL of 0.02*M* acetate buffer, pH 4.5) was used; coupling took place overnight at 4°C, after which the catalyst beads were sieved off and washed several times with 0.3*M* KCl. Characteristics of the glucoamylase catalyst are summarized in Table 1. For use as the mixed yeast/enzyme catalyst for SSF, the catalyst beads were simply mixed with the previously-cultured yeast floc suspension (*see below*).

Fermentation

Fermentation media were made up in warm tap water using a centrifugal pump as a mixer. Media were made up with 120–240 g/L total sugars. For each 100 g of total sugars (measured as glucose or as total invert sugars) the medium contained 0.8 g malt extract, 0.8 g yeast extract, 2 g ammo-

nium sulfate; 3 g potassium dihydrogen phosphate; 0.2 g magnesium sulfate ($7H_2O$); 0.08 g calcium chloride.

The reactor was a continuously-fed 6-L gas-lift tower fermenter (*1*); details of construction and operation are given elsewhere (*4*). Operation was monitored by appropriate sensors in the main reactor volume (temperature, pH) and by a computer-controlled headspace gas chromatograph system to measure ethanol levels in the reactor (*8*).

All runs were carried out at 33 ± 0.3°C and pH 4.5 ± 0.3. Start-up was usually performed using a synthetic medium (glucose) so as to observe standard behavior before any substrate change. Since the reactor operates with up to 100% retention of particulate biomass (*4*), the concentration of yeast catalyst is built up to working levels (from 50 to 100 g/L dry weight). When a sufficiently high biomass density had been achieved, the glucose medium feed was replaced by the test feedstock.

For the experiments with molasses feedstock, the initial yeast concentration was 60 g/L. For the SSF experiments, the glucoamylase catalyst was mixed into the yeast suspension in the proportion 1 vol glucoamylase suspension (15 g/L) to 20 vol yeast suspension (60 g/L).

RESULTS

Measurements of reactor performance give an indication of the practical biocatalyst stability in the different systems; biocatalyst stability was the main factor limiting the process performance for both the tested substrates, though for different reasons and to different extents. Performance was verified by varying the sugar content of the feed and the dilution rate and measuring the substrate conversion and ethanol production; so far as possible, the aim was to maximize the conversion yield (ethanol out/sugar in).

Molasses Substrate

Table 2 summarizes average values of the process variables measured at different concentrations of molasses in the input feed. In each case these are trial-and-error values, obtained as a result of searching for combinations of the two accessible variables—sugar feed concentration and dilution rate—giving the best attainable substrate conversion and stable operation. In practical terms, these values represent virtual maxima of stable performance because of the effect of molasses on the biocatalyst (yeast floc) stability; those effects are also summarized in Table 2. The reactor performance depends on its retention of the yeast flocs, and as Table 2 shows, the stability of these flocs depends on the input substrate strength.

The effect appears proportional to the input sugar concentration, but this is because the input of nonsugars (salts and other nonfermentables)

Table 2
Bioreactor Performance Data Obtained with Molasses Substrate

Sugar concentration, g/L^{-1}	Dilution rate, h^{-1}	Cell concentration, g/g	$Y_{P/S}$	Productivity, g/Lh	Sugar conversion, %	Particle characteristics
125.4	0.20	88	0.44	9.8	89.8	Dense flocs
152.0	0.16	70	0.40	9.6	84.0	Some smaller flocs
166.0	0.12	56	0.40	7.0	83.0	Flocs and free cells
225.0	0.05	50	0.23	2.1	82.9	Free cells, few flocs

in the feedstock is proportional to the input sugar *and is unaffected by the conversion of fermentable sugars into ethanol.* Thus, the inhibitory effect of these substances in the feed is independent of the fermentation and is strictly proportional to feed strength. As feed strength is increased, the sustainable concentration of biocatalyst in the reactor falls; the dilution rate giving reasonable conversion must be decreased, so that productivity falls dramatically. At the higher level of molasses feed concentration, the fermentation yield (ethanol formed/sugar consumed) also falls, indicating that the yeast is under severe stress (*11*). In practical terms, stable operation is effectively limited to input sugar levels not greater than ca 150 g/L, and preferably about 120 g/L. However, within these limits the reactor operates rather well, as the comparison in Table 4 (discussed below) reveals.

Dextrinized Starch Substrate

The performance in SSF with the mixed biocatalyst (yeast particles + glucoamylase particles) was quite different. The sedimentation properties of the yeast particles and the immobilized glucoamylase beads were very similar, and both were satisfactorily retained in the reactor. In this system, further growth of the yeast particles is limited either by ethanol inhibition or by rate-limiting substrate supply—that is, by the effectiveness of the glucoamylase in hydrolysis of the dextrinized starch feedstock. Excess substrate as such had little or no effect, but the degree of conversion was strictly limited by the level of glucoamylase catalyst in the reactor.

Under batch conditions, the mixed biocatalyst performed well; in repeat batches with reuse of the biocatalyst over a 15-d period there was an overall reduction of about 10% in the rate of ethanol fermentation.

However, under continuous operation, although the amount of the glucoamylase catalyst remained, at best, the same (and in practice tended to fall), the amount of yeast tended to increase at a rate determined by the prevailing ethanol level (*4*). The rheology of the suspension becomes limiting at a combined biocatalyst level of about 100 g/L dry weight (*4*). If,

Table 3
Effect of Dilution Rate on Stability
in the Mixed Biocatalyst (SSF) System

Days operation	1 d	3 d
Dilution rate 0.04 h^{-1}		
unreacted sugars in effluent, g/L	<2	6
ethanol in effluent, g/L	57	55
reactor productivity, g/L h	2.3	2.2
catalyst concentration, g/L	64	67
glucose transformation rate, g/g h	0.16	0.15
Dilution rate 0.08 h^{-1}		
unreacted sugars in effluent, g/L	12	37
ethanol in effluent, g/L	56	52
reactor productivity, g/L h	4.5	4.2
catalyst concentration, g/L	59	68
glucose transformation rate, g/g h	0.43	0.24
Dilution rate 0.15 h^{-1}		
unreacted sugars in effluent, g/L	24	42
ethanol in effluent, g/L	56	43
reactor productivity, g/L h	9.0	6.9
catalyst concentration, g/L	66	78
glucose transformation rate, g/g h	0.39	0.25

through yeast growth, this limit is reached, adjustment can be made only by the simultaneous bleeding-out of both biocatalysts. This reduces the rate of starch hydrolysis and consequently the prevailing ethanol level; in turn, the specific growth rate of the yeast increases (4), and the imbalance between glucoamylase and yeast is exacerbated. The resultant instability is most marked when the reactor is operated at lower ethanol levels; at ethanol levels resulting in a minimal growth rate, the onset of instability is deferred.

These effects can be followed in the results summarized in Table 3. At a low dilution rate (0.04 h^{-1}) the reactor is fairly stable, with only a slight deterioration after 5 d (data not shown); feedstock conversion is very satisfactory. At a higher dilution rate, productivity is higher, but substrate conversion is less good and shows significant deterioration, along with a marked build-up of yeast, within 3 d; at a dilution rate of 0.15 h^{-1} deterioration and yeast build-up are very marked.

DISCUSSION

Performance data for the reactor, obtained so far as possible at the working limits for a stable operation, are summarized in Table 4, together with the comparable data for this reactor run on a synthetic glucose-based

Table 4
Bioreactor Characteristics on Different Feedstocks

Characteristic	Feedstock		
	Glucose[a]	Molasses	Starch
Dilution rate, h^{-1}	0.42	0.16	0.04
Biocatalyst concentration, g/L	95	65	69[b]
Substrate input, g/L	157	155	144
Residual sugars, g/L	19	25	5
Effluent ethanol, g/L	54	60	55
Substrate conversion, %	88	84	98
Productivity, g/Lh	22	10	2

[a]Data from Ref. (4).
[b]Mixed yeast plus immobilized glucoamylase.

feedstock (4). They show that, with the molasses feedstock, stable operation can be combined with a useful productivity, provided that the constraints imposed by the destabilizing effects of the molasses on the biocatalyst yeast particles are recognized. A reasonable sugar conversion (84%) and ethanol output (at 60 g/L) can be sustained at a productivity that is practically useful, even though it is less than half that of the same reactor on the synthetic feedstock.

With the dextrinized starch feedstock in the SSF mode, the limitations are more severe, and the system is only marginally practical. At low dilution rates—or in a batch system with recycled catalyst—substrate conversion is very good and the results compare favorably with other reports (*12*); however, at the higher throughput rates needed to give better productivity, the intrinsic instability of the system becomes its dominant feature.

ACKNOWLEDGMENT

H. F. de Castro is indebted to the Brazilian Research Council CNPq for research support through the execution of this work.

REFERENCES

1. Bu'Lock, J. D. (1984), UK Patent 2,066,843B.
2. Comberbach, D. M. and Bu'Lock, J. D. (1984), *Biotechnol. Lett.* **6,** 129–134.
3. Smart, N. J. (1979), PhD thesis, University of Manchester (UK).
4. Bu'Lock, J. D., Comberbach, D. M., and Ghommidh, C. (1984), *Chem. Eng. J.* **29,** B9–B24.
5. Williams, P. D. (1983), PhD thesis, University of Manchester (UK).
6. Maiorella, B. L., Blanch, H. W., and Wilke, C. R. (1984), *Biotech. Bioeng.* **26,** 1003–1025.

7. Klim, C. R. (1983), *Enzyme Microb. Technol.* **5**(3), 103–113.
8. Comberbach, D. M. and Bu'Lock, J. D. (1983), *Biotech. Bioeng.* **25**, 2503–2508.
9. Maeda, H., Chen, L. F., and Tsao, G. T. (1979), *J. Ferment. Technol.* **57**(3), 238–243.
10. Castro, H. F. de (1985), PhD thesis, University of Manchester (UK).
11. Castro, H. F. de and Bu'Lock, J. D. (1988), *Proc. 8th International Biotechnology Symposium*, Paris.
12. Fukushima, S. and Uamade, K. (1983), *Proc. Conference on Advances in Fermentation*, London.