0273-2289/88/1700-0295/$03.60

Production of Propionic Acid Using a Xylose Utilizing *Propionibacterium*

M. J. T. CARRONDO,* J. P. S. G. CRESPO, AND M. J. MOURA

Biochemical Engineering Laboratory, Department of Chemistry, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2825 Monte da Caparica, Portugal

ABSTRACT

The kinetics of *P. acidipropionici* (ATCC25562), a xylose-utilizing rumen microorganism, was studied to assess its use for propionic acid production from wood hydrolyzates.

Propionic acid has been shown to have a stronger inhibitory effect than acetic acid, with the undissociated acid form being responsible for the majority of the inhibitory effect. Thus, in batch tests with pH controlled at 6.0, the propionic acid concentration reaches 25 g/L and the acetic acid 7 g/L. Xylose uptake rate is dependent on the specific growth rate and glucose concentration.

An immobilized cell columnar reactor at very high product yields (80%) proved adequate for propionic production. At cell concentrations of ~95 g/L with high product concentration, volumetric productivities of 2.7 g/L·h were obtained in ultrafiltration cell recycle systems.

Index Entries: Propionic acid; production from *Propionibacterium acidi-propionici*; xylose utilization, kinetics of; immobilized cell system (ICR), production of propionic acid by; tangential ultrafiltration cell recycle reactor (UFR), production of propionic acid by; high cell concentration system for propionic acid production.

INTRODUCTION

Propionic acid production via fermentation has been advocated for use as a grain preserver and antifungal agent for feeds, a plasticizer, and

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

as a herbicide (*1*). Weight yields are greater than those for ethanol fermentation, and the conversion by hydrogenation and dehydration to propylene gives a one third greater weight yield than conversion of ethanol to ethylene (*2*).

Propionic acid bacteria, namely *P. freundenrichii,* and especially subsp. *shermanii* have been used in some industrial processes for vitamin B_{12} production. Advantages of this organism include (*1*) decreased contamination (since propionate is in itself a bacteriostatic and fungistatic agent), and (2) low energy requirements, since the majority of the fermentation is run under anaerobic conditions for cell growth. *P. acidipropionici* (also known as *P. pentosaceum*) has already been assessed by Prof. Gaddy's group (*3*) as a potential propionic acid producer under immobilized conditions. Playne (*4*), has also studied propionic fermentation, comparing results of *Propionibacterium* with *Veillonella parvula,* an organism that cannot use carbohydrates but uses lactate, pyruvate, and succinate.

MATERIAL AND METHODS

Strain

The organism utilized in all the studies, *Propionibacterium acidi-propionici,* was obtained from the American Type Culture Collection (ATCC 25562) in a freeze-dried form.

Media

Conservation media consists of a standard nutrient containing peptone (1%), yeast extract (1%), and phosphate buffer (.15*M*, pH 7). The carbon source was a mixture of glucose:xylose (3:1) at 25 g/L.

Magnesium and manganese salts were added (.01% and .001%, respectively), and also agar powder (3%), to provide for solid medium tubes that were inoculated in depth, incubated for 36 h at 37°C and kept at 4°C. Transfer was made every two months.

During assays, the organism was kept in a liquid medium (same conditions as for conservation but without agar powder) and transferred every 48 h. The flasks were kept constantly shaking at 37°C.

The carbon source (glucose + xylose, 3:1, 2.5%) was separately sterilized and added to the flasks by injection with sterilized needles.

Inoculum Preparation

Seed cultures, grown for 24–48 h, were used for the inoculation. The organism was kept and prepared for inoculation with 2.5% glucose + xylose; transfer was made by sterile injection.

Analytical Methods

Biomass Determination

Cell concentration was determined using the optical density measured at 540 nm and comparing with a calibration curve (optical density vs cell dry wt). On all readings, uninoculated medium was used for zero correction.

Acetic and Propionic Acid Determination

The organic acids propionic and acetic were determined by gas chromatography using a United Technologies Packard GC-439 chromatograph. A glass column 1.8 m long and 2 mm internal diameter, packed with 10% SP 1200 1% H_3PO_4 on chromosorb waw 80/100 mesh was used. Nitrogen was used as a carrier gas (at 40 mL/min). An oven temperature of 115°C, injector temperature of 130°C and detector of 135°C were maintained throughout.

Prior to injection, all samples were centrifuged at 6000 rpm for 10 min, and the cells discarded. To ensure total conversion of all acids to a nondissociated form, an oxalic acid solution (.5*M*) was used for dilutions. A sample volume of .6 μL was used. After every three injections .6 μL of oxalic acid solution was injected in order to avoid ghosting or tailing of the peaks.

An integrator (Shimadzu CR3-A) on-line with the chromatograph was used to determine the composition of each sample. A two-point calibration, external standard method was used. Standards were equally diluted with oxalic acid solution.

Glucose and Xylose Determination

Glucose was determined enzymatically using a Yellow Springs (YSI 27) instrument. Xylose determination was carried out on a HPLC chromatograph (Waters Model-410). A Sugarpack column was used with demineralized water as the solvent phase at a flow rate of .5 mL/min. A two-point, external standard method was used, achieving very good linearity. Prior to injection, samples were centrifuged at 6000 rpm and the cells discarded. Samples were then filtered through Sartorius membranes (.45 μm) and diluted with demineralized water.

Viscosity Assay

Broth viscosity was estimated with a couette viscometer (Rheomat 15 TFC) at 37°C.

Batch Reactors

All reactors were sterilized at 121°C for 20 min. Sugar solutions, separately sterilized, were added after cooling to room temperature. Inocula were 3% (v/v), 24–48 h old.

Batch Reactors without pH Control

Borosilicate glass flasks (100 mL) were used with a 70 mL working volume. Flasks were sealed with rubber stoppers and aluminum capsules with a central sampling point allowed aseptic conditions during fermentation.

Substrate addition and inoculation were made by injection under aseptic conditions. During fermentation, flasks were kept in a thermostatic batch with longitudinal shaking, at 37°C and 140 strokes/min.

Batch Reactors with pH Control

A polibatch battery was used, with 2 L fermenters (Setric 2*M*), which allowed temperature, pH, and mixing speed control. Substrate and inocula were added by sterile connection with glass connectors. Temperature was kept at 37°C, revolution speed at 150 rpm, and pH at 6.00, by addition of diluted ammonia (13.4*M*).

Chemostat Reactors

Setric Fermenters (2 L and 7 L) were used, operated as a CSTR. Temperature, revolution speed, and pH were controlled in the same way as for the batch reactors.

Immobilized Cell Reactor

A columnar reactor was used built from a plexiglass tube, 3.16 cm in internal diameter, 47 cm in height, and .3 cm wall thickness. The reactor was closed at both ends by plexiglass plates; the bottom was fixed and the top removable, to allow for filling and washing of the column. A NPT Swagelok (¼ in. connection) was fixed at the center of each plate for feeding (bottom) and effluent discharge (top).

Five sampling ports, at 8 cm intervals along the column, were made using ⅛ in. NPT Swagelok, screwed to the column and sealed with rubber septa. The bed was made up of 4 mm glass beads covered with a solution of agar (1%) and gelatin (25%), which was subjected to attack with glutaraldehyde solution (3%) prior to sterilization with ethylene oxide. The system (including medium reservoir, reactor, pumps, and so on) was kept in a temperature-controlled chamber at 37°C.

Cell Recycle Ultrafiltration System

This system consisted of a Setric fermenter (2 L), working on-line with two parallel ultrafiltration modules (CARBOSEP M 6, manufactured by SFEC) with zirconium oxide membranes on a carbon support. The ultrafiltration modules were tubular, with seven separate type M 6 membranes, containing a maximum of 1.5 L of medium. The filtration surface was .158 m^2 with a cutoff of 500,000 d. The second module was used in case of flow interruption by cell accumulation on the working module

and allowance was made for rinsing and change under sterile conditions (Fig. 1).

RESULTS AND DISCUSSION

Fermentation Kinetic Studies

Batch reactor studies were undertaken to describe product inhibition conditions during fermentation and to assess glucose-xylose consumption kinetics. The inhibitory effects were clearly demonstrated by data obtained from tests conducted using (*1*) external acid addition to the broth in a concentration range of 0 to 20 g/L, (*2*) pH control. In both cases, acetic or propionic acid inhibitory effects can be described by hyperbolic model of the type (*5*)

$$\mu_m^i/\mu_m^o = K_p/(K_p + P_a^m) \quad (1)$$

Where μ_m^o and μ_m^i are the maximum specific growth rates, without and with product addition to the fermentation media, resp.; K_p is a parameter related to the resistance to the added product; and m, the exponent of the added product concentration P_a, represents the degree of strain tolerance to each product. Data fitting yielded the following results

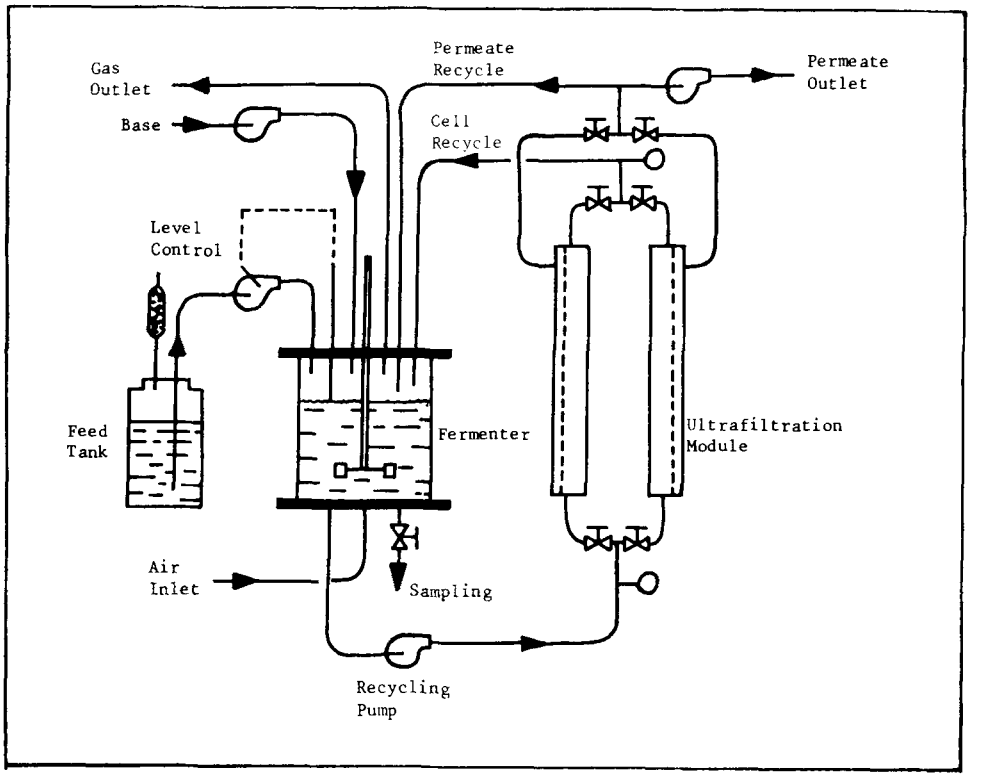

Fig. 1. Schematic representation of the ultrafiltration cell recycle system.

1. Acetic acid $\mu_m^i/\mu_m^o = 2943.8/(2943.8+P_a^{2.179})$ $r = .92$ (2)

2. Propionic acid $\mu_m^i/\mu_m^o = 76.1/(76.1+P_a^{1.209})$ $r = .98$ (3)

The inhibitory effect is much stronger with propionic acid, being very small for acetic acid (large K_p) at potential process concentrations. A set of batch tests at 2, 3, 5, 8.5, and 12% initial glucose concentrations was also conducted. Since glucose consumption curves and final glucose utilization were the same in all the experiments, fermentation was not controlled by the initial glucose concentration within the range tested.

The inhibitory effect due to propionic acid produced during fermentation is shown in Fig. 2, where the specific growth rate, μ, and the specific production rate, ν, are plotted against propionic acid concentration. As can be seen, specific growth rate tapers off at somewhat lower concentrations than the specific production rate, i.e., production still takes place at higher concentrations than those sufficient to stop growth. The relationship between μ and P can be linearly described up to 4 g/L of propionic acid and exponentially thereafter.

$$P < 4 \text{ g/L} \qquad \mu = .211 - .0355\ P \tag{4}$$

$$P > 4 \text{ g/L} \qquad \mu = 1.2804 \exp(-.726\ P) \tag{5}$$

On the other hand, ν and P can be described exponentially throughout the whole range

$$\nu = .366 \exp(-3.328\ P) \tag{6}$$

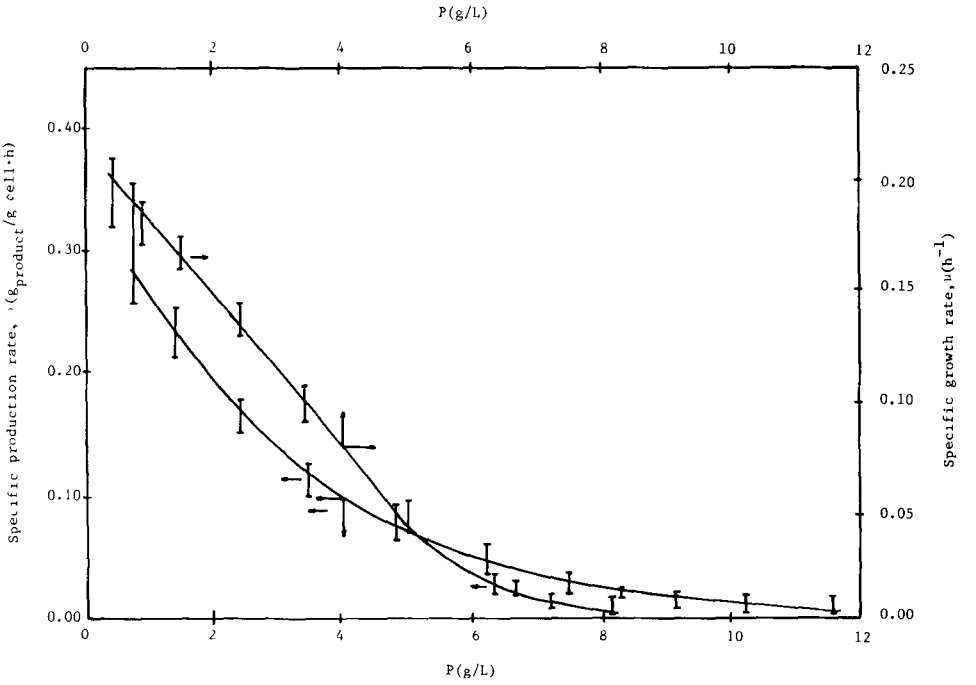

Fig. 2. Specific rates of growth and propionic acid production.

Comparing the inhibitory effect of added versus produced propionic acid, one can conclude that a stronger inhibition occurs in the case of the produced acid which is also apparent in other fermentation systems (*6*).

To assess xylose utilization, a set of batch studies was performed using glucose/xylose ratios of 3:1, 1:1, and 1:3 at total sugar concentrations of 50 g/L. Xylose utilization was low (maximum of 2.5 g/L at 75% xylose) and no diauxic growth seems to take place. Fermentation curves for experiments carried out at 25 and 50% of xylose are similar and do not differ from curves obtained from fermentations using glucose as the only substrate. However, if glucose becomes limiting, as happens at 75% xylose concentration, organic acid production is dramatically affected, reaching a maximum of only 6.5 g/L of propionic acid and 1.9 g/L of acetic acid.

Acids were produced at a ratio close to 3:1 propionic/acetic (mol basis) as compared to 2:1 theoretical ratio, obtained from the stoichiometry (*7*).

$$1.5\ C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2COOH + CH_3COOH + CO_2 + H_2O \quad (7)$$

Since propionic acid has a higher cost, this shift is favorable and should be increased as much as possible. Table 1 shows the average performance obtained in batch assays with no substrate limitation. These results fit well with those obtained by Hendricks et al. (*8*). The cell mass yield coefficient is somewhat high for a facultative anaerobe, probably lowering the product yields. At the low final pH values obtained in these experiments (in spite of the presence of phosphate buffer), propionic and acetic acid are essentially in their undissociated forms. Thus, transport through the cell membrane is easier. Consequently, its inhibitory effect becomes rather important (*9*).

To overcome this inhibitory effect, batch tests were conducted with pH controlled at 6.0. The results obtained are summarized in Table 2. Total sugar concentrations of 75 g/L and different sugar ratios were utilized:

Table 1
Batch Tests without pH Control

Maximum specific growth rate, μ_{max}	h^{-1}	.11
Maximum cell concentration, X_{max}	g/L	4.3
Final acid concentration/Propionic acid	g/L	11.0
Acetic acid		3.0
Glucose consumption	g/L	22.0
Total product yield	wt%	63.6
Cell yield, $Y_{X/S}$	g cell/g substrate	.263
Maximum volumetric productivity	g prod./L·h	.19
Maximum cell mass productivity	g cell/L·h	.13
P/A mol ratio		3.0
Final pH		4.7

Table 2
Batch Tests Under pH Controlled at 6.0

Sugar concentration, g/L		75 glu[a]	54 glu + 18 xyl[a]	38 glu + 37 xyl	19 glu + 55 xyl
Maximum specific growth rate, μ_{max}, h^{-1}		.143	.140	.130	.127
Maximum cell concentration, X_{max}, g/L		7.2	7.35	6.25	5.9
Final acid	Propionic	24.6	22.9	16.6	9.6
concentration, g/L	Acetic	6.9	7.6	5.9	4.0
Consumption,	Glucose	61.3	54	38	19
g/L	Xylose	—	4.9	8.5	12.3
Total product yield, wt%		51	52	48	44
Maximum volumetric	Propionic	.24	.24	.22	.18
productivities, g/L·h	Propionic + Acetic	.29	.30	.29	.25
P/A mol ratio		2.9	2.4	2.3	2.0

[a]glu—glucose, xyl—xylose

glucose only, 3:1 glucose/xylose, 1:1 glucose/xylose, and 1:3 glucose/xylose. Higher acid concentrations and volumetric productivities were apparent as compared to the uncontrolled batch tests (Figs. 3 and 4). A clearer picture for glucose and xylose consumption is depicted in Fig. 5 where glucose concentration in the broth and xylose consumption are plotted vs fermentation time. It is clear that both sugars can be fermented simultaneously but with a higher uptake rate for glucose. However, there is no uptake of xylose during the first 23 h and the fermentation kinetics are initially close to that observed with glucose alone. After glucose exhaustion, no xylose consumption takes place, which agrees with the fact that fermentation does not proceed with xylose only. From Fig. 5, xylose and glucose consumption rates were calculated for the time periods when these were maximum and approximately constant. These results are presented in Table 3.

It seems evident that there is an inhibition of xylose uptake by an excess of glucose. So the xylose consumption rate varies inversely with glucose concentration. However, care must be taken in interpreting this data. For instance, for the 3:1 glucose/xylose ratio essay and when glucose concentration gets small, the microorganism is no longer growing exponentially and no increase in xylose consumption rate is apparent. As glucose:xylose ratios are decreased to 1:1 and 1:3, xylose consumption rate shoots up, as the glucose concentration range is lowered (within the exponential growth phase). These results suggest that the xylose uptake

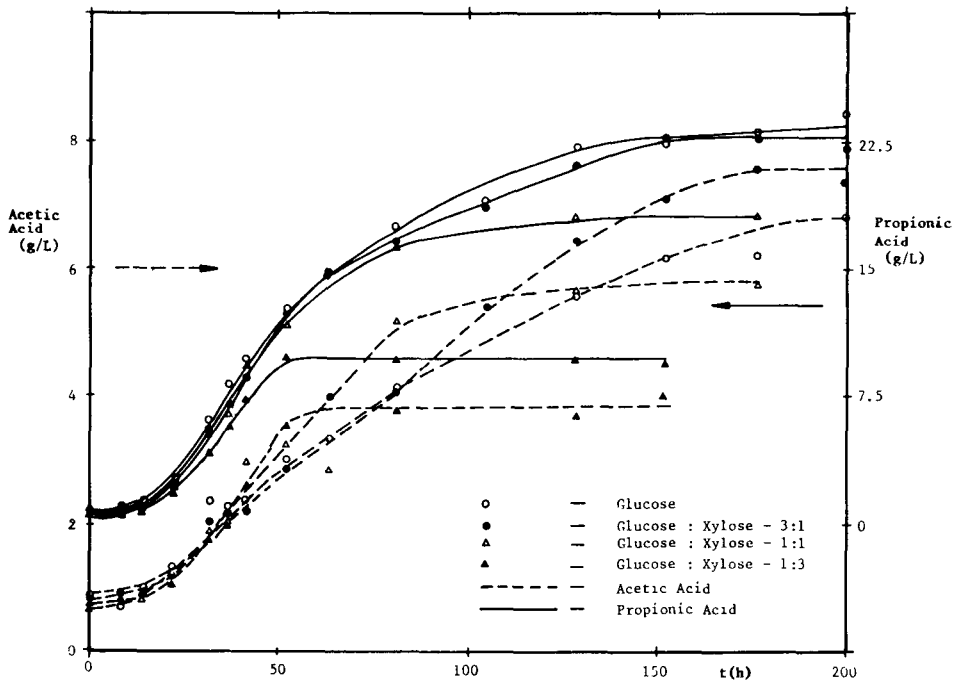

Fig. 3. Acetic and propionic acids production in batch reactor at pH = 6.00.

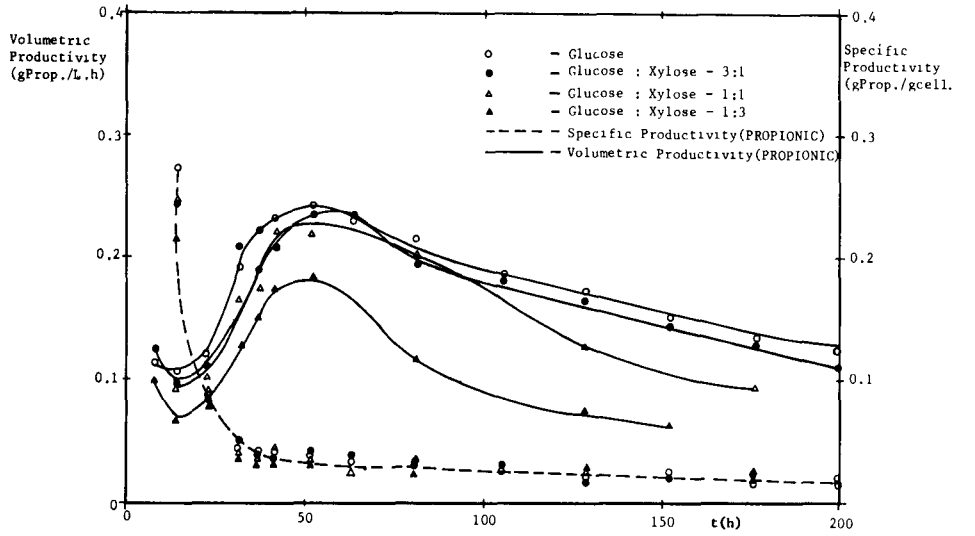

Fig. 4. Specific and volumetric productivities in batch reactor at pH = 6.00.

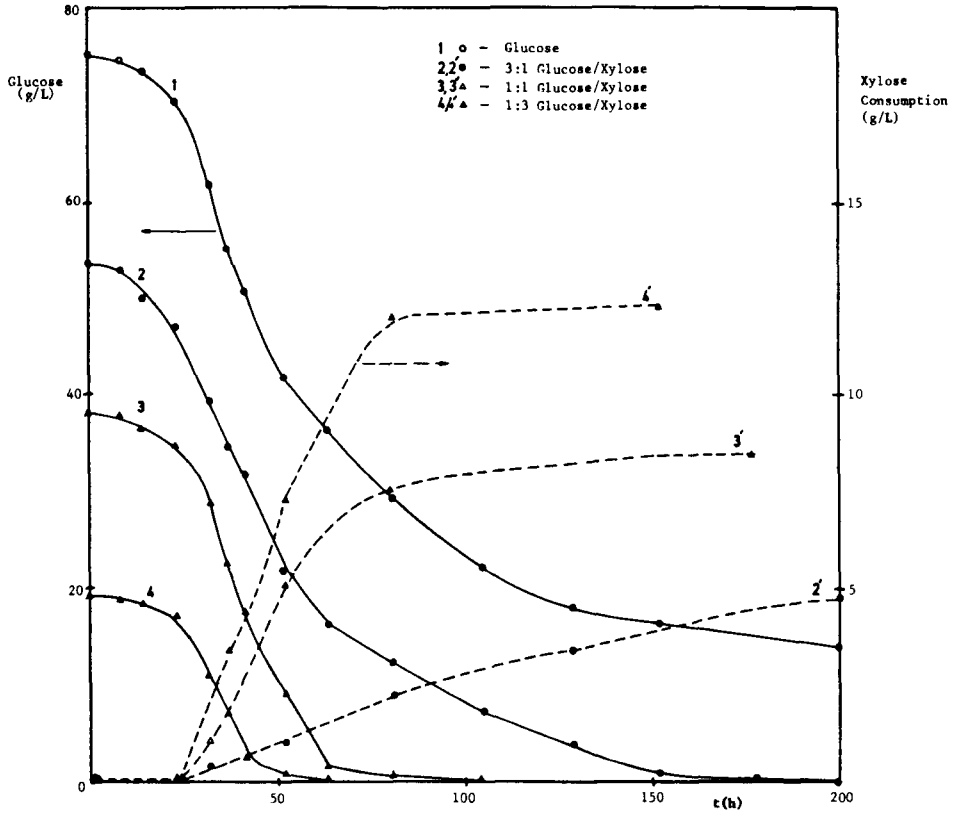

Fig. 5. Glucose concentration and xylose consumption in batch reactor at pH = 6.00.

Table 3
Sugar Consumption Rates in Batch Tests, pH 6.0, 23 to 52 h

	3:1 glu/xyl[a]	1:1 glu/xyl	1:3 glu/xyl
Xylose consumption rate, g/L·h	.04	.18	.21
Glucose consumption rate, g/L·h	.87	.87	.56
Range of glucose conc., g/L	47–22	35–9	17–1

[a]glu/xyl = glucose/xylose

rate, in the presence of glucose, might be described by a model of the type

$$- 1/X \, dC_{xyl}/dt = f(\mu, C_{glu}) \quad (8)$$

CONTINUOUS REACTOR STUDIES

Three continuous reactor types were assessed at 3:1 glucose/xylose ratios: continuous stirred tank reactor (CSTR) (*1*), immobilized-cell columnar reactor (ICR) (2), and a CSTR with ultrafiltration cell-recycle (UFR) (3).

CSTR tests conducted without pH control and with pH control at 6.0 (feed sugar concentrations of 30 g/L and 75 g/L, respectively), were performed, essentially to compare its performance with the batch reactors. Figures 6, 7, and 8 show the results obtained. The CSTR at pH 6.0 washed out at 6 to 7 h, in agreement with the maximum specific growth rate of .140 h^{-1} obtained in batch tests under similar conditions. Maximum xylose consumption was low at 1.3 g/L, while glucose concentration in the broth remained high. Maximum volumetric productivities were reached at 17.6 h (D = 0.057 h^{-1}) at .42 g/L·h and .57 g/L·h for pro-

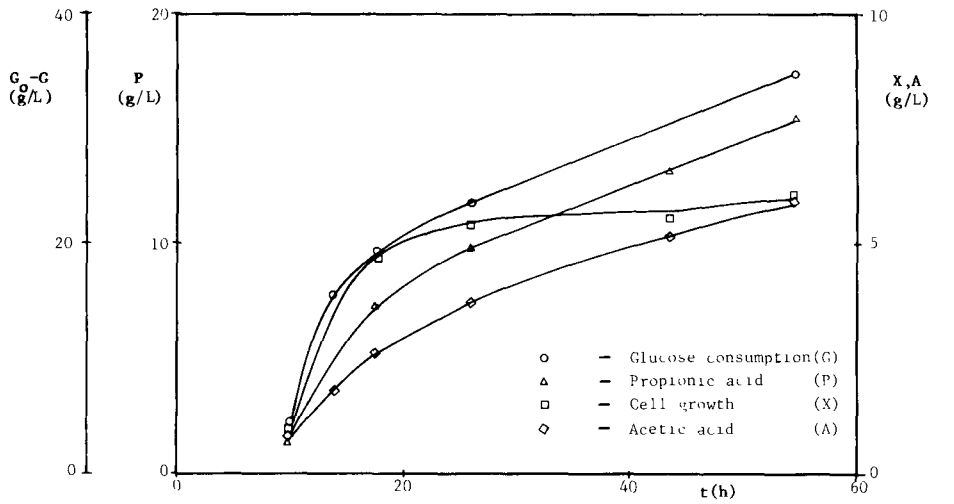

Fig. 6. Glucose consumption, cell growth, propionic and acetic acids production in CSTR at pH = 6.00.

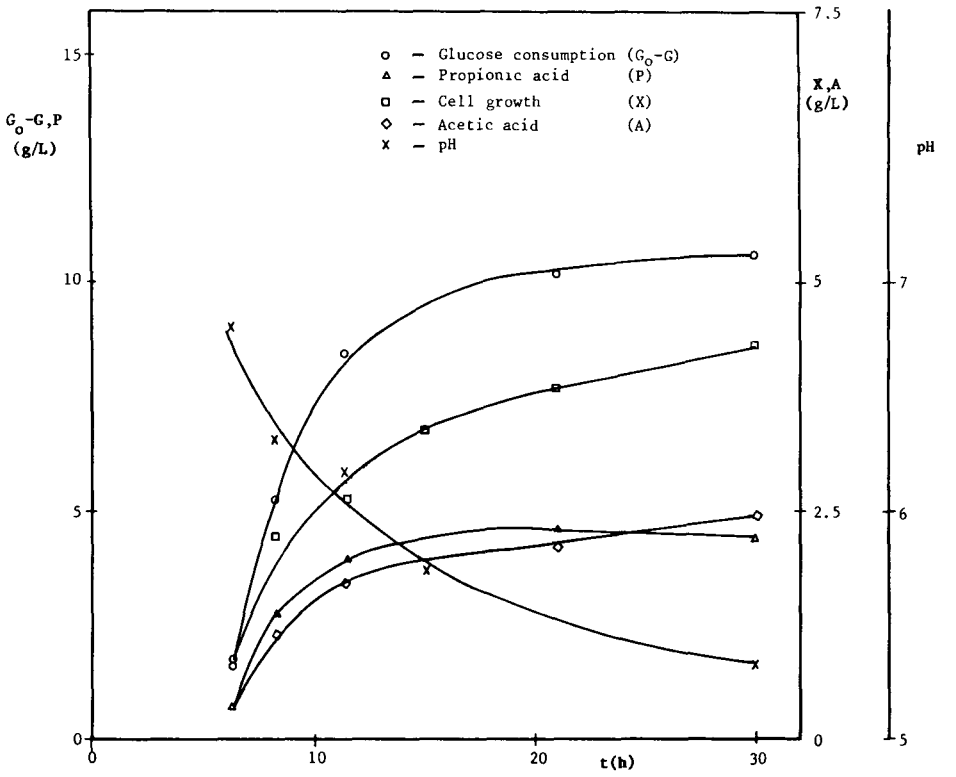

Fig. 7. pH, glucose consumption, cell growth, propionic and acetic acids production in CSTR without pH control.

pionic and total acids, respectively. Nevertheless acid concentrations were low at 7.3 g/L propionic acid and 2.7 g/L acetic acid. Maximum total yield of 61% was obtained at 42 h with 13.4 g/L propionic acid and 5.2 g/L acetic acid at a total volumetric productivity of .45 g/L·h. It is worth mentioning that the propionic to acetic mol ratio remains almost constant at 2.2, a value closer to the theoretical ratio than obtained under batch conditions.

As expected, the CSTR without pH control yields very low acid concentrations—in spite of good volumetric productivities (*see* Figs. 7 and 8). This effect becomes more noticeable when the pH drops below 6, at approximately 12 h retention time. At such a retention time, volumetric and specific productivities are almost identical with and without pH control at 6 (*see* Fig. 8). Furthermore, productivities drop much faster without pH control.

ICR tests were carried out over a period of six months. During its operation, degassing of CO_2 (produced during fermentation) became a major problem. It reduced the useful reactor volume and the immobilized cells-nutrient broth contact. Since the column was operated without pH control, a strong inhibitory pH value of 5.2 was reached within the column. With these conditions full use of the column proved difficult to

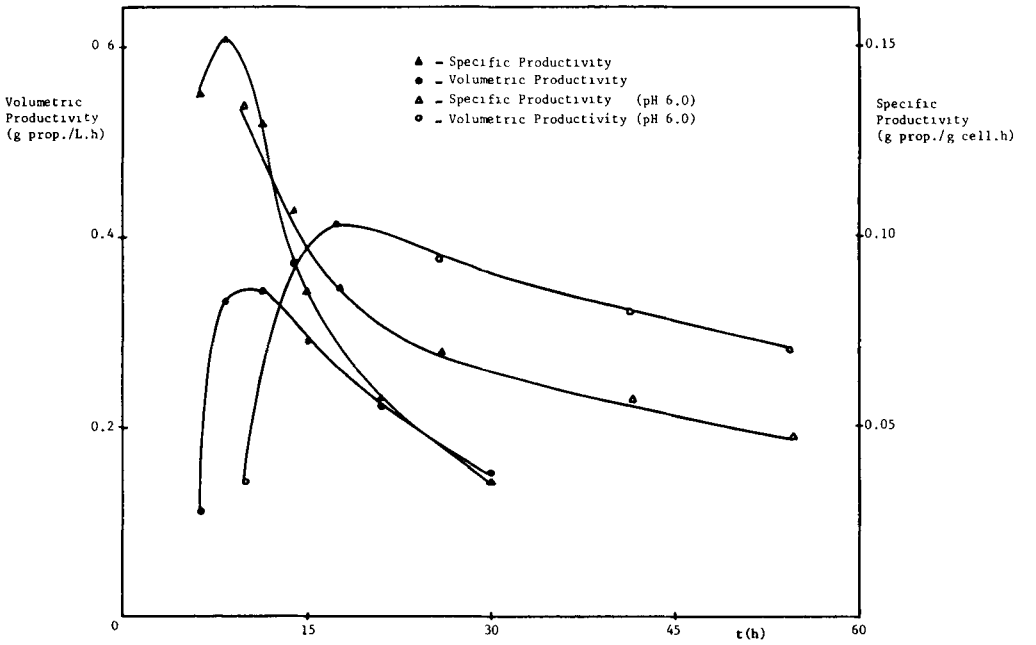

Fig. 8. Specific and volumetric productivities (propionic acid) in CSTR without pH control (▲,●) and with pH = 6.00 (△,○).

achieve. Starting from 25 g/L total sugars and at a residence time of 16.5 h, final concentrations of 9.5 g/L propionic acid and 1.2 g/L acetic acid, i.e., a 6.4 mol ratio, were obtained at productivities of .95 g/L·h for propionic acid and 1.03 g/L·h for total acids. Product yields were high at 80% since less substrate was utilized for cell growth purposes. Xylose consumption was low at 1 g/L, a result to be expected since glucose concentration was low only at very low pH.

UFR tests were performed with pH control at 6.0 and a total sugar concentration of 50 g/L. Figure 9 presents the fermentation curves. The system was initially operated in a batch way and after that period, it was run continuously for two dilution rates. At .09 h^{-1} (11.1 h residence time), 18.5 g/L propionic acid and 4.3 g/L acetic acid were achieved at propionic and total acids volumetric productivities of 1.6 and 2.0 g/L·h. Increasing the dilution rate to .12 h^{-1} (8.33 h residence time) increased propionic and total acids volumetric productivities to 2.2 and 2.7 g/L·h, with final acids concentrations almost unchanged at 18 g/L propionic acid and 4 g/L acetic acid. Blanc (*10*), using lactose substrates, increased propionic productivity to 2.15 g/L·h at a dilution rate of .3 h^{-1}, but with a low propionic concentration in the effluent (9 g/L). The results we obtained are encouraging, since a good compromise between productivities and acids concentration was achieved. This compromise is important and will be reflected on downstream process costs for product recovery. The mol ratio for the acids was 3.5 and the yield for total acids was 59%. The productivity reported here is 14 times greater than productivity obtained in

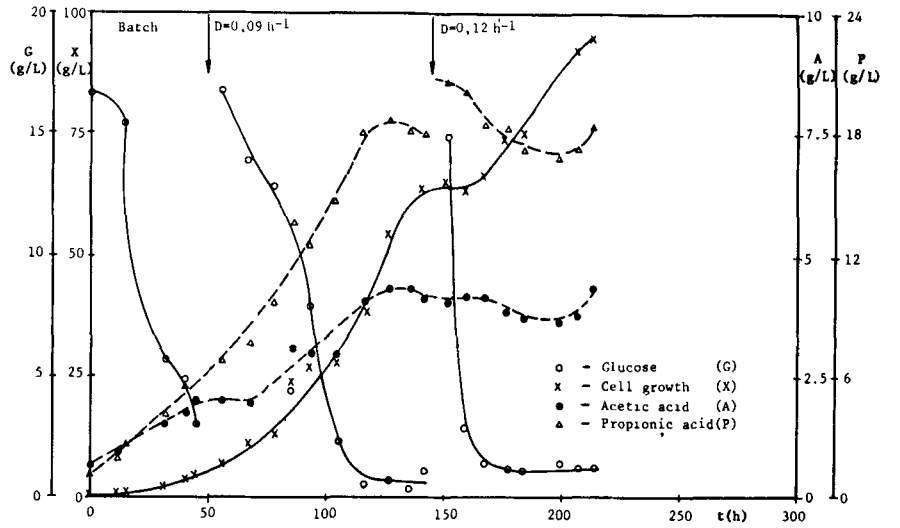

Fig. 9. Glucose consumption, cell growth, propionic and acetic acids production in UF system.

the batch reactor without pH control and 9 times that in the batch with pH control at 6.0.

Since the final cell concentration reached 95 g/L in the system, i.e., 14 times higher than in the batch with pH control, but the productivity was only 9 times that in the batch, it can be concluded that a loss of cell activity occurred. This might be explained by a decay of cell viability because of shear stress developed along the system, the rheological conditions associated with such high cell concentrations, and accumulation of inhibitory cometabolites. The system still presents a Newtonian rheology behavior, with a final apparent viscosity of 13.3 $mP_a \cdot s$.

Figure 10 shows the apparent viscosity versus cell concentration. This relationship can be described by a model of the type

$$\eta_{app} = 1 + AX^n \tag{9}$$

Data fitting yielded the following results.

$$\eta_{app} = 1 + 6.156 \times 10^{-4} X^{2.186} \quad r = 0.987 \tag{10}$$

Performance results obtained from continuous fermentation systems are summarized on Table 4.

CONCLUSIONS

In order to achieve higher xylose consumption, a better understanding of the xylose uptake rate as a function of specific growth rate and glucose concentration must be sought. Given the sugar concentrations to be expected in real situations (3:1 glucose/xylose), a glucose consumption

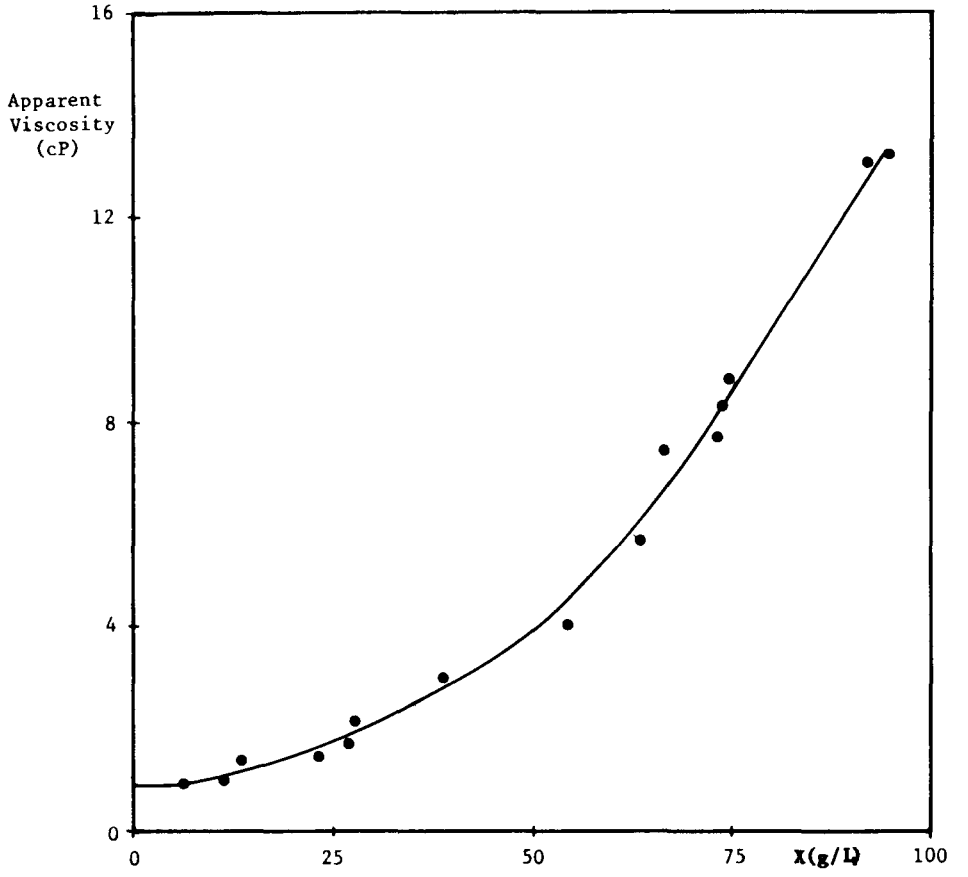

Fig. 10. Apparent viscosity vs cell growth in UF system.

rate three times higher than xylose would be ideal. Such a result was obtained in the batch tests for the opposite sugar ratio of 1:3 (*see* Table 3).

To develop a model, the utilization of a chemostat fed with nutrient broth containing xylose and with a separate glucose solution feed seems to be appropriate (fed batch situation). The use of different dilution rates would allow the study of the influence of specific growth rate on the kinetics of xylose utilization, while on-line glucose control would allow keeping its concentration within a desirable range.

The ICR reactor presented higher yields than any of the other reactor types tested; pH control and degassing are important requirements to be improved for better operability of this reactor type.

Since the price of propionic acid is higher than that of acetic acid, a larger P/A mol ratio is desirable. UFR and ICR systems, possibly as a result of larger populations of nongrowing cells, seem most appropriate for the purpose. These high-cell-concentration reactors might perform even better if operated under extractive/product removal conditions and/or pH control to decrease inhibition.

Table 4
Performance Comparison in Continuous Fermentation Systems

		CSTR pH 6.0	CSTR	ICR	UFR
Maximum volumetric productivity, g prod./L·h	Propionic	.42	.34	.95	2.20
	Total	.57	.49	1.03	2.70
Corresponding acid concentration, g/L	Propionic	7.3	3.9	9.5	18.0
	Acetic	2.7	1.7	1.2	4.0
Yield for total acids, w/w%		61	66	80	59
Percentage of theoretical maximum yield[a]		79	86	104	77
P/A mol ratio		2.2	1.9	6.5	3.5

[a]Assuming Stoichiometry given by Eq.7.

ACKNOWLEDGMENTS

J. P. Crespo carried out some of the glucose batch tests without pH control at J. Gaddy's laboratory at the University of Arkansas, Fayetteville, and the UFR at G. Goma's laboratory at INSA, Toulouse, France. We hereby express our recognition for these ongoing collaborations.

M. J. Moura acknowledges a research studentship awarded by Junta National de Investigação Científica e Tecnológica, Lisboa (JNICT). We acknowledge: financial support of JNICT under contract No. 720.85.74; US Program in Science and Technology Cooperation, Washington, DC, Grant No. 936-5542-G-SS-4003-00, as well as the sponsorship of IPE-Investimentos e Participações do Estado, SARL and Portucel, E.P.

NOMENCLATURE

t	Time, h
G	Glucose concentration, g/L
G_o	Initial glucose concentration, g/L
X	Biomass concentration, g/L
A	Acetic acid concentration, g/L
P	Propionic acid concentration, g/L
P_a	Product concentration added to broth, g/L
μ	Specific growth rate, h^{-1}
μ_m	Maximum specific growth rate, h^{-1}
ν	Specific production rate, g product/g cell·h
$Y_{X/S}$	Biomass yield, g cell/g substrate
$Y_{P/S}$	Product yield, g product/g substrate
D	Dilution rate, h^{-1}
η_{app}	Apparent viscosity, m P_a·s

REFERENCES

1. Anon (1981), Chemical Economics Handbook, SRI International, Stanford, CA.
2. Humphrey, A. E. (1977), *Chem. Eng. Prog.* **73** (5), 85–91.
3. Clausen, E. C., and Gaddy, J. L. (1984), *Chem. Eng. Prog.* **80** (12), 59–63.
4. Playne, M. J. (1985), *Propionic and Butyric Acids* in M. Moo-Young, Ed., *Comprehensive Biotechnology*, Pergamon, London (1985).
5. Mota, M. J. (1985), Docteur Ingenieur Thesis, INSA, Toulouse.
6. Nagodawithana, T. W., Steinkraus, K. H. (1976), *J. Appl. Environ. Microbiol.*, **31,** 158.

7. Allen, S. H. G., Kellermeyer, R. W., Stjernholm, R., Wood, H. G. (1964), *J. Bacteriol.* **87,** 171–187.
8. Hendricks, B., Korus, R. A., Heimsch, R. C. (1985), *Biotechnol. Bioeng. Symp.* **17,** 241–245.
9. Samson, F. E., Katz, A. M., Harris, D. L. (1955), *Arch. Biochem. Biophys.* **54,** 406–423.
10. Blanc, P. (1986), Docteur Ingenieur Thesis, INSA, Toulouse.