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Production of L-lactic Acid and Oligomeric Compounds from Apple Pomace by Simultaneous Saccharification and Fermentation: A Response Surface Methodology Assessment

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The potential of apple pomace for lactic acid production by simultaneous saccharification and fermentation (SSF) was evaluated. The effects of the cellulase to solid ratio (CSR), the liquor to solid ratio (LSR), and the β-glucosidase to cellulase ratio (BCR) on the kinetics of lactic acid generation were assessed, and a set of mathematical models was developed to reproduce and predict the lactic acid concentration of fermentation broths. Operating at low cellulase and cellobiase charges (1 FPU/g and 0.25 IU/FPU, respectively) and short reaction times (10 h), SSF media containing 27.8 g of lactic acid/L were obtained with a volumetric productivity of 2.78 g/Lh. Material balances showed that the SSF processing of 100 kg of dry apple pomace results in the production of 36.6 kg of lactic acid, 18.3 kg of oligomeric carbohydrates (which can be used as ingredients for functional foods), 8.4 kg of microbial biomass, and 8 kg uronic acids.

KEYWORDS: Apple pomace; L-lactic acid; oligosaccharides; *Lactobacillus rhamnosus*; SSF

INTRODUCTION

Several million metric tons of apple pomace (the main solid waste generated in the production of cider and apple juice) are produced annually worldwide (*1*). In Spain, the cider-making industries produced >20000 metric tons of apple pomace in 2003.

Pomace is mainly used as a feed component (a low added-value application) and in pectin manufacture, but according to Kennedy et al. (*1*), the ideal use for apple pomace has yet to be found. In this context, bioconversion of apple pomace into lactic acid is an interesting possibility. Lactic acid has a number of applications in food technology (as acidulant, flavor, and preservative), pharmaceuticals, and chemicals (*2*). The world market for lactic acid is growing every year, and its current production is about 150 million pounds per year. The worldwide market is expected to grow between 10 and 15% per year (*3*). Lactic acid is mainly produced by fermentation of enzymatic hydrolysates from starch extracted from grain. A number of different substrates have also been used for the production of lactic acid by bacteria. Lactic acid manufacture by fermentation of a pure sugar results in lower purification costs, but it is economically unfavorable because of the high substrate cost. Alternatively, waste products from agroindustries and forestry (including whey, molasses, starch from wastes and lignocellulosic materials) can be utilized as raw materials (*2*). In addition, further cost cuttings can be achieved if no chemical pretreatments have to be implemented and low enzyme charges can be used.

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Apple pomace shows advantages as a substrate for lactic acid production, including (i) a high content of free glucose and fructose, which are excellent carbon sources for lactic acid production (*2*); (ii) a high content of polysaccharides (cellulose, starch, and hemicelluloses), which can be enzymatically hydrolyzed at low enzyme charges; (iii) the presence of other compounds (e.g., monosaccharides other than glucose and fructose, di- and oligosaccharides, citric acid, and malic acid) that can be metabolized by lactic bacteria (*4*); and (iv) the presence of metal ions that could limit the cost of nutrient supplementation for fermentation media.

On the other hand, during the simultaneous saccharification and fermentation (SSF) process, a variety of oligomers (derived from cellulose, hemicelluloses, and starch) are simultaneously generated in the medium. This kind of compound has potential in food industries, where they can be used as food ingredients with functional properties (*5*).

This paper deals with the experimental assessment of the lactic acid production from apple pomace by SSF in media containing using cellulase−cellobiase mixtures and *Lactobacillus rhamnosus* cells. The production of potentially valuable process byproducts (oligomers derived from polysaccharides and pectin, microbial biomass) was also considered in experiments, to explore the possibility of developing a multiproduct process.

MATERIALS AND METHODS

Raw Material. Nine samples of apple pomace were collected (two samples per week) in a local cider-making factory (Sidrería Gallega, Chantada, Lugo, Spain) during the harvesting and processing period. To avoid their degradation, all samples were submitted to a drying

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stage at 60 °C for 24 h. Dried samples were milled to a particle size in the range of 0.2−0.5 mm and mixed to obtain a representative and homogeneous lot, which was stored at −18 °C. Aliquots from this lot were employed in experiments.

Analytical Methods. *Solvent Extraction.* Soxhlet extraction with 80% ethanol (operating at a liquor to solid ratio of 30 g/g) led to extractives (E) and to an alcohol-insoluble fraction (AIF), which were analyzed separately.

Analysis of the Extractives (Fraction E). Extractives were assayed for dry residue by oven-drying at 105 °C (ISO method 638:1978), and their contents of sugars, oligosaccharides, L-malic acid, and uronic acids were measured. Monosaccharides were quantified by HPLC using a 1100 series Hewlett-Packard chromatograph fitted with a refractive index detector (temperature, 50 °C). Other analysis conditions were as follows: column, ION-300 (Transgenomic, Inc.); mobile phase, 0.003 M H_2SO_4; flow, 0.6 mL/min. In HPLC chromatograms, glucose was eluted separately, whereas fructose, xylose, mannose, galactose, and malic acid were eluted together in a second peak, and rhamnose and arabinose were eluted together in a third peak. Additional determinations of glucose, fructose, and saccharose were performed using the Boheringer-Mannheim enzymatic kit reference no. 10716260035. The L-malic acid content was measured using the Boheringer-Mannheim enzymatic kit reference no. 10139068035. Glucooligosaccharides, xylooligosaccharides, and arabinooligosaccharides were measured by HPLC on the basis of the increase in sugar concentration caused by acid posthydrolysis of liquors (*5*). The results were corrected for fructose decomposition, because this sugar was partially degraded during posthydrolysis. Uronic acids were determined using the method of Blumenkrantz and Asboe-Hansen (*6*).

Analysis of the Alcohol-Insoluble Fraction (AIF). The AIF was subjected to quantitative acid hydrolysis (TAPPI T13m method), and liquors were assayed by HPLC as described above. The results allowed the determination of the contents of glucose polymers (here denoted as glucan), hemicellulosic polysaccharides, and acetyl groups. Xylose, galactose, and mannose making part of the hemicelluloses were quantified separately from other hemicellulosic sugars (arabinose and rhamnose). Uronic acids were determined using the same method cited above. Neutral-detergent fiber (NDF), acid-detergent fiber (ADF), and acid-detergent lignin (ADL) were determined according to the methods of Goering and Van Soest (*7*). Starch was determined enzymatically (Boheringer-Mannheim kit reference no. 10207748035).

Other Analyses. Moisture and ashes were determined according to methods ISO 638:1978 and ISO 776, respectively.

Elemental nitrogen was determined with a Thermo Finnigan Flash EATM 1112 analyzer, using 130 and 100 mL/min of He and O_2 and an oven temperature of 50 °C. All determinations were made in triplicate. Protein content was obtained by multiplying the elemental N content by 6.25.

Metals and elemental P were analyzed using an atomic absorption spectrometer 220 Fast Sequential. When necessary, samples were digested with 5 mL of HNO_3 65%, 1 mL of H_2O_2 30%, and 0.5 mL of HF 40% in a Microwave Labstation mls 1200 mega.

Microorganism, Medium, and Inoculum Preparation. The *Lactobacillus rhamnosus* CECT-288 (ATCC-9595) strain employed in this work was obtained from the Spanish Collection of Type Cultures (Valencia, Spain). Cells were grown on plates using the complete medium, which contained 20 g of glucose/L, 4 g of yeast extract/L, 8 g of meat extract/L, 10 g of peptone proteose/L, 5 g of sodium acetate/L, 2 g of triammonium citrate/L, 2 g of K_2HPO_4/L, 0.4 g of $MgSO_4 \cdot 7H_2O$/L, 0.055 g of $MnSO_4 \cdot H_2O$/L, 1 g of polysorbate 80/L, and 14 g of agar/L at 37 °C for 24 h. Inocula were prepared by transferring cells from plates to 100 mL of MRS broth medium placed in 250 mL Erlenmeyer flasks. After 12 h of shaking (150 rpm, 37 °C), 5 mL of the medium was transferred to the same amount of MRS medium, which was incubated again for 12 h under the same conditions and then used as inocula for SSF experiments. Biomass concentration in concentrated inocula (17 g of dry cells/L) was measured by absorbance readings at 600 nm.

Enzymes. Enzyme concentrates ("Celluclast 1.5L" cellulases from *Trichoderma reesei* and "NS50010" β-glucosidase from *Aspergillus niger*) were kindly provided by Novozymes (Madrid, Spain). The cellulase activity of Celluclast 1.5L concentrates was measured by the filter paper assay (*8*), and the activity was expressed in terms of filter paper units (FPU). The β-glucosidase activity of NS50010 concentrates was measured by the PNPG assay (*9*) and reported as International Units (IU). Amylase activity in enzyme concentrates was measured according to the method of Murado et al. (*10*) and expressed as enzyme units (EU). The cellulase activity of the Celluclast 1.5L concentrate was 170 FPU/mL, and the β-glucosidase and amylase activities of the NS50010 concentrate were 575 IU/mL and 1664 EU/mL, respectively.

SSF. SSF experiments were carried out in 250 mL Erlenmeyer flasks (working volume, 100 mL) placed in orbital shakers (150 rpm). SSF media were prepared by mixing the desired amounts of apple pomace, water, nutrients (MRS broth without glucose), and calcium carbonate to neutralize the lactic acid produced. The amounts of the components were calculated for each experiment depending on the particular operational conditions (cellulase to solid ratios in the range of 1−12 FPU/g, liquor to solid ratios in the range of 12−20 g/g, and β-glucosidase to cellulase ratios in the range of 0.25−5 IU/FPU). Suspensions containing the desired amounts of water, apple pomace, and calcium carbonate were autoclaved separately from the nutrients (121 °C, 20 min) and thermostated at 45 °C. SSF experiments were started by inoculation (10 mL) and enzyme addition. At given fermentation times, samples from the media (0.5 mL) were taken and centrifuged at 5000 rpm for 5 min. Aliquots of the supernatants were withdrawn for the various analyses. Triplicate experiments were carried out in the central point of the experimental domain to assess the influence of the experimental error.

A SSF experiment was carried under selected conditions in a Biostat B-Braun fermenter with automatic pH control. At the end of the experiment, a sample was withdrawn and analyzed for its contents of monosaccharides, lactic acid, acetic acid, and oligosaccharides using the methods cited above. Viable cell concentration was also determined after serial dilutions (up to 10^{-7}) with NaCl 0.5% w/w. Triplicate plates for each dilution were then inoculated with 100 μL and incubated at 37 °C for 2−4 days (*11*). Man-Rogosa−Sharpe (MRS) agar media were used for cultivation. Cell concentrations were calculated from plates having a discernible number of colonies (20−200). Following the methods cited above, the conversion factor of 1.52×10^{-6} g of microbial biomass/cfu was determined for inocula and used to calculate the amount of microbial biomass in the final fermentation broth.

Fitting of Data and Modeling. The experimental data were fitted to the proposed models using commercial software (Microsoft Excel from Microsoft).

RESULTS AND DISCUSSION

Composition of the Raw Material. Table 1a shows the chemical composition of apple pomace extractives, which accounted for 38.5% of the oven-dry apple pomace weight. Fermentable sugars (monosaccharides and saccharose) and malic acid, all of them suitable carbon sources for lactic acid bacteria, accounted for 87.4% of the oven-dry weight of extractives (odb), confirming that this byproduct has a high potential as a raw material for lactic acid production.

The AIF (accounting for 61.5% of dry apple pomace) was mainly made up of cell wall polysaccharides (cellulose, hemicelluloses, and pectin), lignin, and starch. **Table 1b** shows the composition of AIF. Glucan (including cellulose, starch, and other glucose-containing polysaccharides) was the major component (41.9% of the oven-dry AIF weight).

The polysaccharides containing xylose and/or mannose and/or galactose (e.g., arabinoxylan and arabinogalactan) accounted for 13.9 wt % of oven-dry AIF. Minor percentages of arabinose- and rhamnose-containing polymers (9 wt % AIF, oven-dry basis) were also present in the raw material.

To get further information on the AIF glucan, cellulose (25.1%) was measured by fiber analysis (a methodology already

Table 1. Composition of the Raw Material

	wt % oven-dry basis
(**a**) Composition of Extractives (38.5% Oven-Dry Weight of Apple Pomace)	
saccharose	5.8
glucose	19.7
fructose	48.3
XMnGa[a]	4.4
L-malic acid	3.2
arabinose and rhamnose	6.0
glucooligosaccharides	3.4
xylooligosaccharides	3.0
arabinooligosaccharides	0.2
uronic acids	3.4
(**b**) Composition of the Alcohol-Insoluble Fraction (61.5% Oven-Dry Weight of Apple Pomace)	
glucan	41.9
starch	14.4
cellulose	25.1
$XMnGa_n$[b]	13.9
AR_n[c]	9.0
acid detergent lignin (ADL)	15.2
acetyl groups	1.1
uronic acids	15.3
(**c**) Other Components	
ashes	1.49
protein (6.25 × N)	3.8
P	72.6×10^{-3}
K	69.3×10^{-2}
Mn	52.8×10^{-5}
Fe	33.8×10^{-4}
Mg	40.3×10^{-3}

[a] XMnGa, xylose, mannose, and galactose. [b] $XMnGa_n$, polysaccharides made up of xylose, mannose, and galactose. [c] AR_n, polysaccharides made up of arabinose and rhamnose.

employed in previous papers on apple pomace, see refs *1* and *12*), whereas the starch content (14.4%) was determined enzymatically.

Considering the amount of glucose determined after total hydrolysis, it can be inferred that 2.5% of AIF is made up of non-cellulose, non-starch polysaccharides.

Finally, **Table 1c** shows the ash, protein, K, Mn, Fe, and Mg contents of the raw material.

SSF Experiments. The main objective of this study was to assess the lactic acid production by SSF of apple pomace in media containing cellulase−cellobiase mixtures and *L. rhamnosus*. For this purpose, the response surface methodology was employed to develop mathematical models describing the effect of the operational conditions on the lactic acid production.

Table 2 summarizes the experimental plan, including the fixed variables and their values, the independent variables [cellulase to solid ratio (CSR), liquor to solid ratio (LSR), and β-glucosidase to cellulase ratio (BCR)] and their variation ranges, and the dependent variables (fitting parameters and oligomer concentrations, see below).

Table 3 shows the set of experiments, the structure of which corresponded to an incomplete, factorial, centered experimental design. The dimensionless, normalized independent variables (x_1, x_2, and x_3), with variation ranges (−1, 1) are linearly related to the dimensional independent ones (CSR, LSR, and BCR). Similar experimental designs have been used in the literature to study the enzymatic saccharification of starch (*13*) and to optimize the poly-b-hydroxybutyrate production (*14*).

As a representative example, **Figure 1** shows the concentration profiles corresponding to experiments 1, 4, 8, and 12 of **Table 3**.

Table 2. Experimental Variables Involved in the Study of Simultaneous Saccharification and Fermentation

variable	definition and units	nomenclature	value or range
fixed	temperature (°C)	T	45
	pH	pH	5–6
	final reaction time (h)	t	18
	initial biomass concn (g/L)	X_0	1.7
independent	cellulase to solid ratio (FPU/g)	CSR or x_1	1–12
	liquor to solid ratio (g/g)	LSR or x_2	12–20
	β-glucosidase activity to cellulase activity ratio (IU/FPU)	BCR or x_3	0.25–5
dependent	initial volumetric productivity to initial lactic concn ratio (h^{-1})	P'_0 or y_1	
	max lactic acid concn (g/L)	P_m or y_2	
	glucooligosaccharides concn at 10 h (g/L)	GOS_{10} or y_3	
	xylooligosaccharides concn at 10 h (g/L)	XOS_{10} or y_4	
	arabinooligosaccharides concn at 10 h (g/L)	AOS_{10} or y_5	

Table 3. Operational Conditions Assayed in SSF Experiments Carried (Expressed in Terms of the Dimensional Variables CSR, LSR, and BCR and in Terms of the Dimensionless Variables x_1–x_3)

	dimensional, independent variables			dimensionless, independent variables		
expt	CSR (FPU/g)	LSR (g/g)	BCR (IU/FPU)	x_1	x_2	x_3
1	1	12	2.625	–1	–1	0
2	1	16	0.25	–1	0	–1
3	1	16	5	–1	0	1
4	1	20	2.625	–1	1	0
5	6.5	12	0.25	0	–1	–1
6	6.5	12	5	0	–1	1
7	6.5	16	2.625	0	0	0
8	6.5	16	2.625	0	0	0
9	6.5	16	2.625	0	0	0
10	6.5	20	0.25	0	1	–1
11	6.5	20	5	0	1	1
12	12	12	2.625	1	–1	0
13	12	16	0.25	1	0	–1
14	12	16	5	1	0	1
15	12	20	2.625	1	1	0

In SSF experiments, two stages could be observed. In the initial one, fermentation was the limiting step of the overall kinetics (owing to the comparative faster rate of enzymatic hydrolysis), leading to the accumulation of glucose and AR. In this period, fructose and glucose were metabolized simultaneously without acetic acid generation. In the second stage, both glucose and fructose concentrations decreased sharply, and lactic acid was generated at high rate, the enzymatic hydrolysis becoming the limiting step. After 8−10 h, glucose and fructose were completely depleted. No acetic acid was generated during glucose and fructose fermentation, whereas AR consumption and acetic acid generation started once glucose was exhausted. Acetic acid generation has been reported for homofermenters under glucose limitation and for growth on non-glucose sugars (*2*).

Mathematical Modeling. Mathematical models able to predict the product concentration as a function to the operational conditions were developed for optimization purposes. The dependence of lactic acid concentration (P) on the fermentation time (t) was interpreted using an equation formally equal to

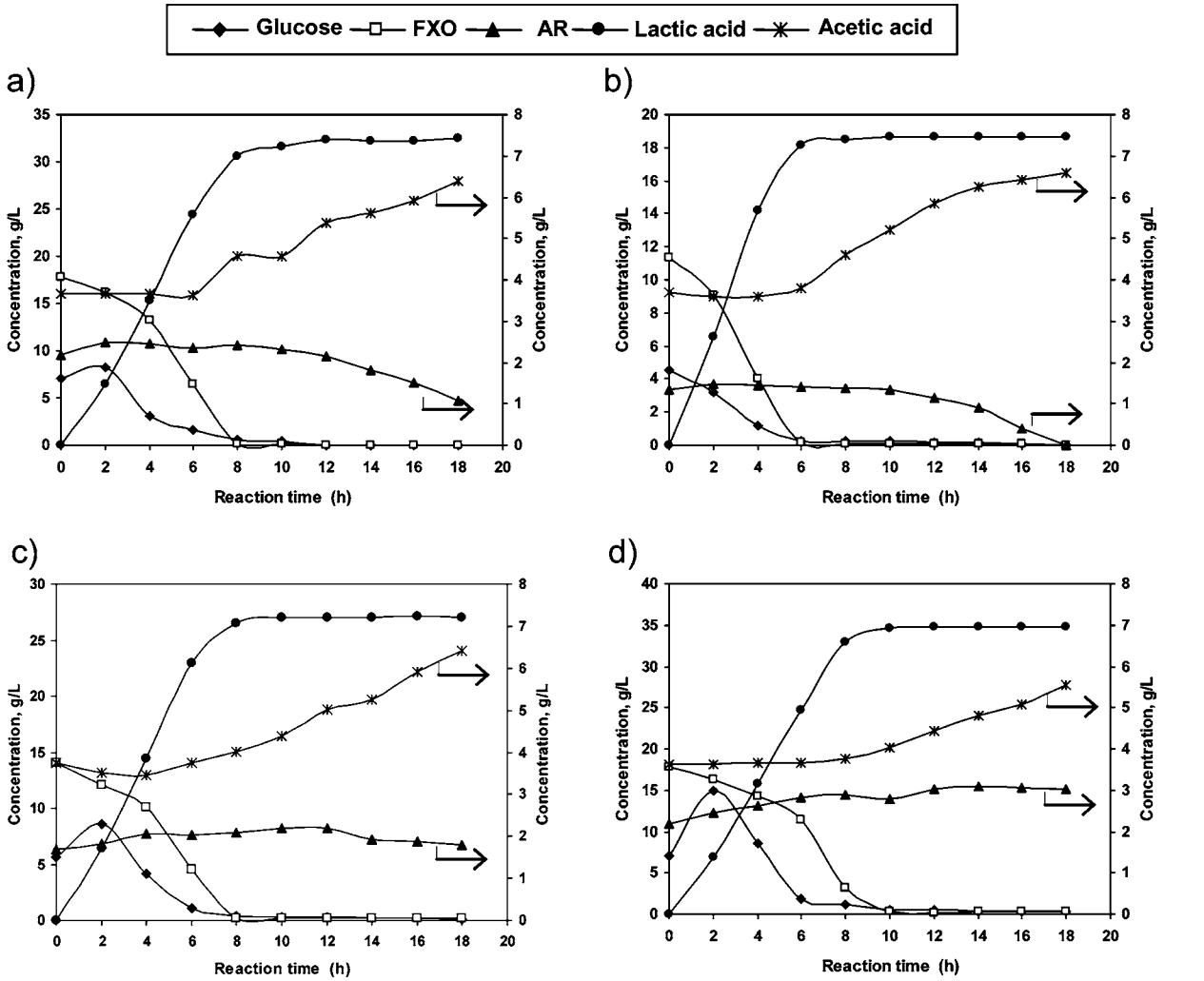

Figure 1. Time course of G (glucose), FXO (fructose, xylose, mannose, and galactose), AR (arabinose and rhamnose), lactic acid, and acetic acid obtained during the SSF assays corresponding to experiments 1 (**a**), 4 (**b**), 8 (**c**), and 12 (**d**).

the one of Moraine and Rogovin, previously used by Mercier et al. (*15*) for correlating data of lactic acid fermentation in batch mode. The expression employed in this work is

$$P = \frac{AP_{\mathrm{m}}\, \mathrm{e}^{P_0' t}}{P_{\mathrm{m}} - A + A\, \mathrm{e}^{P_0' t}} \qquad (1)$$

where P_{m} is the maximum lactic acid concentration, A is a regression parameter with units of concentration, and P_0' is defined as the ratio between the initial volumetric rate of product formation and A. The values determined for the regression were obtained for each experiment for nonlinear fitting of experimental data. The corresponding results are listed in **Table 4**, as well as the R^2 and F values (which varied in the ranges of 0.993−0.997 and 510−1186, respectively), confirming the ability of eq 1 for reproducing the experimental results.

To deduce a generalized expression describing the kinetics of lactic acid production by SSF, A, P_0', and P_{m} were assumed to depend on the operational conditions. A preliminary analysis of data revealed that A was linearly dependent on LSR and independent from CSR and LSR. Therefore, A can be calculated as

$$A = 2.60 - 0.072 \times \mathrm{LSR} \qquad (R^2 = 0.983) \qquad (2)$$

On the other hand, P_0' and P_{m} were correlated with the dimensionless, independent variables (x_1, x_2, and x_3) according to the second-order polynomial expression

$$y_j = b_{0j} + \sum_{i=1}^{3} b_{ij} x_i + \sum_{i=1}^{3} \sum_{k=1}^{3} b_{ikj} x_i x_k \qquad (3)$$

where y_j are the dependent variables ($j = 1-5$), $b_{0j}...b_{ikj}$ are regression coefficients calculated from the experimental results by the least-squares method, and x_i-x_k ($k \geq i$) are the dimensionless, normalized independent variables.

Table 4. Experimental Results Obtained in Experiments 1–15

expt	y_1 or P_0' (h^{-1})	y_2 or P_{m} (g/L)	y_3 or GOS_{10} (g/L)	y_4 or XOS_{10} (g/L)	y_5 or AOS_{10} (g/L)
1	0.689	32.35	3.82	4.69	3.45
2	0.830	21.83	4.92	3.84	2.87
3	0.866	24.59	2.51	3.51	2.58
4	1.020	18.62	2.31	3.00	2.04
5	0.672	34.43	4.78	5.80	4.21
6	0.651	36.09	3.63	5.76	3.46
7	0.787	27.03	2.75	4.28	2.80
8	0.775	27.13	2.74	4.35	2.78
9	0.782	26.98	2.71	4.28	2.86
10	0.914	20.12	3.18	3.67	2.24
11	0.930	21.60	2.40	3.61	2.01
12	0.657	35.04	3.83	5.91	3.82
13	0.807	25.64	3.50	4.82	2.84
14	0.750	27.40	3.59	4.98	2.77
15	0.949	21.94	2.26	3.48	1.72

Table 5. Regression Coefficients and Significance (Based on a *t* Test) and Statistical Parameters Measuring the Correlation and Significance of Models Obtained for Variables y_1–y_5 in the Set of Experiments 1–15[a]

coefficient	y_1 or P_0' (h^{-1})	y_2 or P_m (g/L)	y_3 or GOS_{10} (g/L)	y_4 or XOS_{10} (g/L)	y_5 or AOS_{10} (g/L)
b_{0j}	0.7812*	27.048*	2.74*	4.30*	2.81*
b_{1j}	–0.0303*	1.580*	–0.05	0.52*	0.02
b_{2j}	0.1432*	–6.954*	–0.74*	–1.05*	–0.86*
b_{3j}	–0.0031	0.957*	–0.53*	–0.03	–0.17*
b_{12j}	–0.0100	0.158	–0.02	–0.19**	–0.17**
b_{13j}	–0.0233**	–0.249	0.63*	0.12	0.05
b_{23j}	0.0093	–0.044	0.09***	–0.01	0.13***
b_{11j}	0.0345**	–1.627*	0.23*	–0.23**	–0.14***
b_{22j}	0.0130	1.567*	0.09***	0.19**	0.08
b_{33j}	–0.0023	–0.554**	0.67*	0.21**	0.09
R^2	0.991	0.998	0.996	0.993	0.992
F exptl	64	312	147	84	67
signification level (%)	>99	>99	>99	>99	>99

[a] *, significant coefficients at the 99% confidence level; **, significant coefficients at the 95% confidence level; ***, significant coefficients at the 90% confidence level.

Table 5 lists the regression coefficients ($b_{0j}...b_{ikj}$) obtained by fitting the data shown in **Table 4** to eq 3, as well as the parameters measuring the correlation (R^2) and significance (F) of the models. The values determined for R^2 confirmed the close agreement existing between the experimental and predicted values.

According to **Table 5**, all of the independent variables are significant from a statistical point of view, but CSR and LSR have a higher effect on y_2 (P_m) than BCR. **Figure 2a** shows the calculated dependence of y_2 on the independent variables CSR and LSR for experiments carried out at a BCR = 0.25 IU/FPU ($x_3 = -1$).

Even in experiments performed at very low β-glucosidase loadings (0.25 IU/FPU), maximum lactic acid concentrations in the range of 16.5−34.4 g/L were achieved. CSR had a positive effect on P_m in the first half of its variation range.

Figure 2b shows the calculated dependence of y_2 (P_m) on the independent variables CSR and BCR for experiments performed at LSR = 12 g/g ($x_2 = -1$). BCR affected P_m mainly in the first half of its variation range. Therefore, increases in CSR or BCR above the medium value would not have major effects on P_m, defining a practical range for the enzyme loadings.

It can be noted that the regression coefficients listed in **Table 5** allow the calculation of P_0' and P_m for any operational conditions in the experimental range using eq 3, whereas the value of A can be calculated for the considered LSR value by means of eq 2. Once P_0', P_m, and A are known, the lactic acid concentration (measured by variable P) can be calculated for the desired processing time using eq 1, allowing a generalized calculation procedure. Experimental and calculated lactic acid concentrations showed a close agreement (±5% deviation as an average) for reaction times in the range of 4−14 h (the most important period from a practical point of view), confirming the reliability of the calculation method proposed in this work.

Optimization Considerations. Data calculated from the above mathematical models were employed for making a comparative evaluation of different operational conditions. Considering that reaction times longer than 8−10 h do not increase the final lactic acid concentration and that higher lactic acid concentrations reduce the operational costs, the following discussion is oriented to assess the most favorable enzyme charges (CSR and BCR) for operation at LSR = 12 g/g for 10 h.

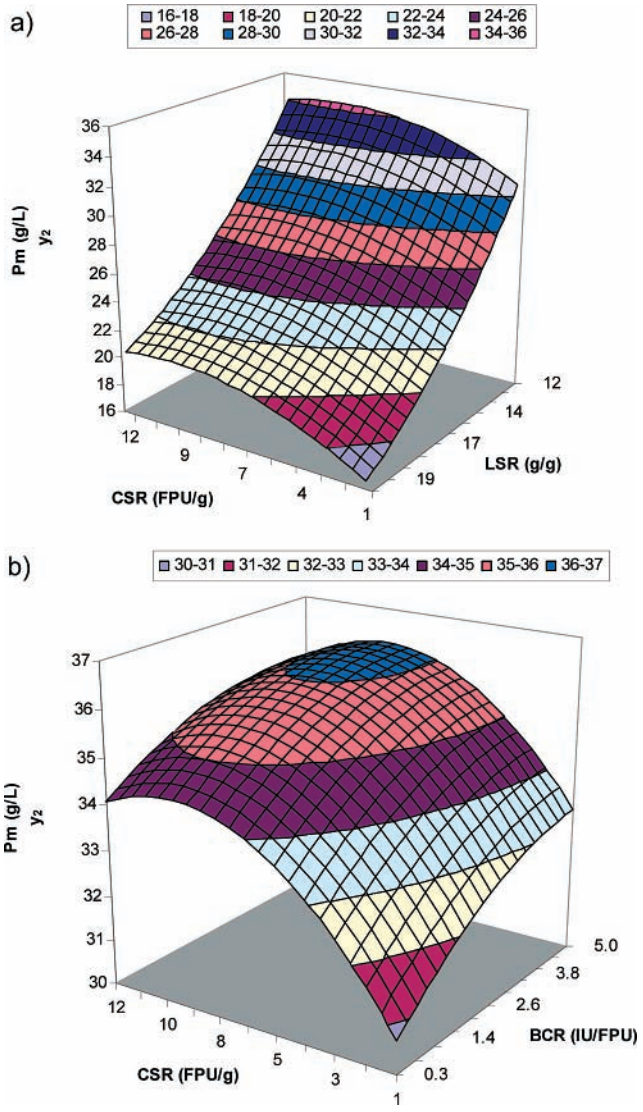

Figure 2. Calculated dependence of P_m (maximum lactic acid concentration) on the operational variables CSR (cellulase to solid ratio), LSR (liquor to solid ratio), and BCR (β-glucosidase activity to cellulase activity ratio): (**a**) BCR = 0.25 IU/FPU; (**b**) LSR = 12 g/g.

Figure 3 shows the dependence of the lactic acid concentration predicted on CSR and BCR for the considered reaction time value. A maximum for variable P (35.0 g/L) was predicted for CSR = 8.7 FPU/g and BCR = 3.81 IU/FPU, with an average volumetric productivity of 3.5 g/Lh. Interestingly, lactic acid concentrations near 34 g/L can be achieved by operation with very low enzyme loadings.

At the end of the SSF process, the medium also contains high amounts of microbial biomass and oligomeric compounds derived from polysaccharides and pectin, a set of products potentially suitable for utilization in food industries. For example, oligosaccharides can be used as ingredients for functional foods (*5*), and *L. rhamnosus* can be considered as a probiotic (*16*), even if this type of application would require the separation of cells from the SSF media.

In this context, the concentrations of glucooligosaccharides (GOS), xylooligosacchardies (XOS), and arabinooligosaccharides (AOS) measured at 10 h (see **Table 4**) were considered

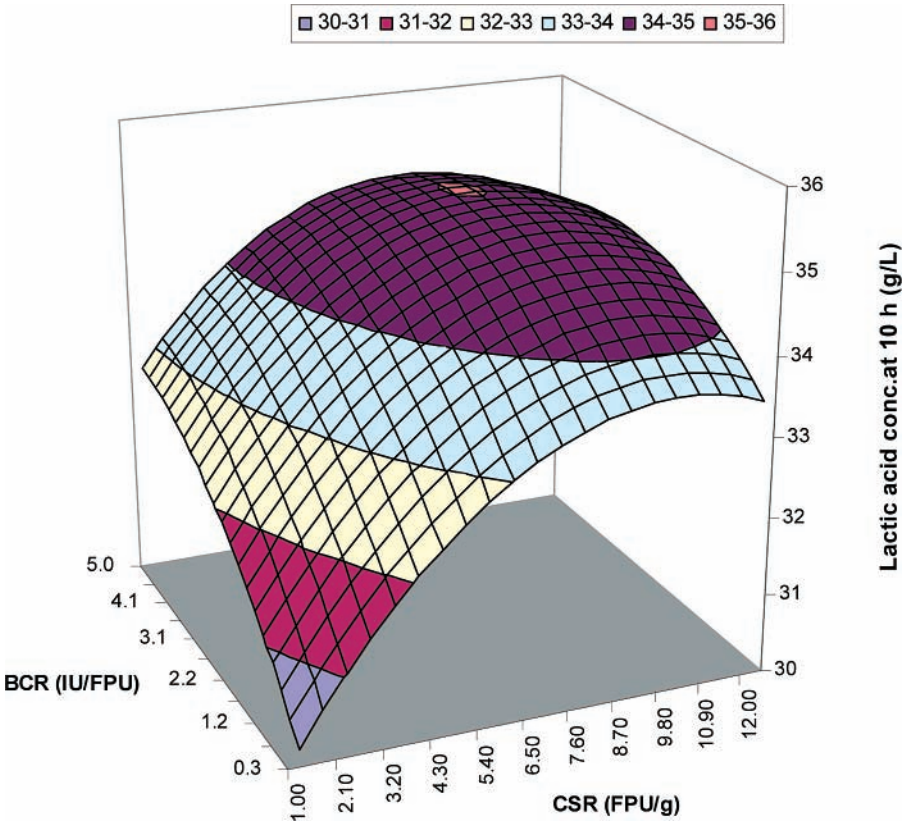

Figure 3. Calculated dependence of lactic acid concentration measured at 10 h of reaction time on CSR (cellulase to solid ratio) and BCR (β-glucosidase activity to cellulase activity ratio) for experiments carried out at liquor to solid ratio = 12 g/g.

as dependent variables for data analysis and correlated with the independent normalized variables using eq 3. **Table 5** lists the regression coefficients ($b_{0j}...b_{ikj}$) obtained as well as the parameters measuring the correlation (R^2) and significance (F) of the models.

Figure 4 shows the calculated dependence of GOS, XOS, and AOS on CSR and BCR for experiments carried out at LSR = 12 g/g and reaction time = 10 h. Operation at low CSR resulted in decreased concentrations of GOS when BCR increased. This behavior is ascribed to the chemical nature of GOS, which were partially made up of starch-derived polysaccharides. The β-glucosidase employed in this work showed amylase activity, and decreased amounts of this enzyme resulted in incomplete hydrolysis of the starchy material. As expected, high enzyme loadings resulted in increased amounts of XOS and AOS as a result of the increased hydrolysis of hemicellulosic polysaccharides.

The maximum total oligosaccharides content (TOS; obtained for the joint contribution of GOS, XOS, and AOS) was predicted for operation at CSR = 8.15 FPU/g and BCR = 0.25 IU/FPU. Under these conditions, the models predicted a TOS concentration of 14.80 g/L together with a lactic acid concentration of 33.6 g/L (see **Figure 3**). The distribution of TOS components was as follows: 4.68 g of GOS/L, 5.94 g of XOS/L, and 4.17 g of AOS/L.

A detailed analysis of the model predictions allowed the identification of operational conditions leading to high concentrations of product and byproducts with low enzyme consumption. Thus, the following data were predicted for operation at CSR = 1 FPU/g and BCR = 0.25 IU/FPU: lactic acid concentration = 30.2 g/L (volumetric productivity, 3 g/Lh), TOS concentration = 14.58 g/L (5.75 g of GOS/L, 4.97 g of XOS/L, and 3.86 g of AOS/L). These operational conditions were considered as the most favorable ones within the experimental domain.

Additional Characterization and Mass Balance. To obtain more information, a SSF experiment was carried out under selected conditions (CSR = 1 FPU/g, LSR = 12 g/g, BCR = 0.25 IU/FPU, and reaction time = 10 h) in a Biostat B-Braun fermenter with automatic pH control. **Table 6** shows the composition of the final fermentation medium (including solid and liquid phases). The experimental values were slightly lower than the predicted for the models under the same conditions. The observed differences are mainly related to the dilution effect caused by the pH control, as the neutralization was performed by adding NaOH with about 5% dilution in volume.

Figure 5 shows the material balance of the studied process calculated from the data included in **Table 6**. Starting from 100 kg of dry apple pomace, 36.6 kg of lactic acid can be obtained after 10 h by SSF operation performed at low charges (1 FPU of cellulase/g and 0.25 IU of β-glucosidase/FPU). A volumetric productivity of 2.78 g of lactic acid/Lh was obtained. These volumetric productivities compare favorably with those reported for other cheap raw materials such as corncob, starch waste, rye, sweet sorghum, wheat, cassava, and potato (*17*).

Considering the simultaneous production of lactic acid from saccharose, fructose, and glucose with a theoretical yield of 1 g of lactic/1 g of sugar, the calculated theoretical conversion yield was 58.4 g of lactic acid/100 g of raw material. Therefore, the operational yield accounted for 63% of the theoretical one. Considering only the major carbon sources (including the monosaccharides present in the raw material and the ones coming from enzymatic hydrolysis), a product yield of 88 kg of lactic acid/100 kg consumed (saccharose + fructose + glucose) was obtained.

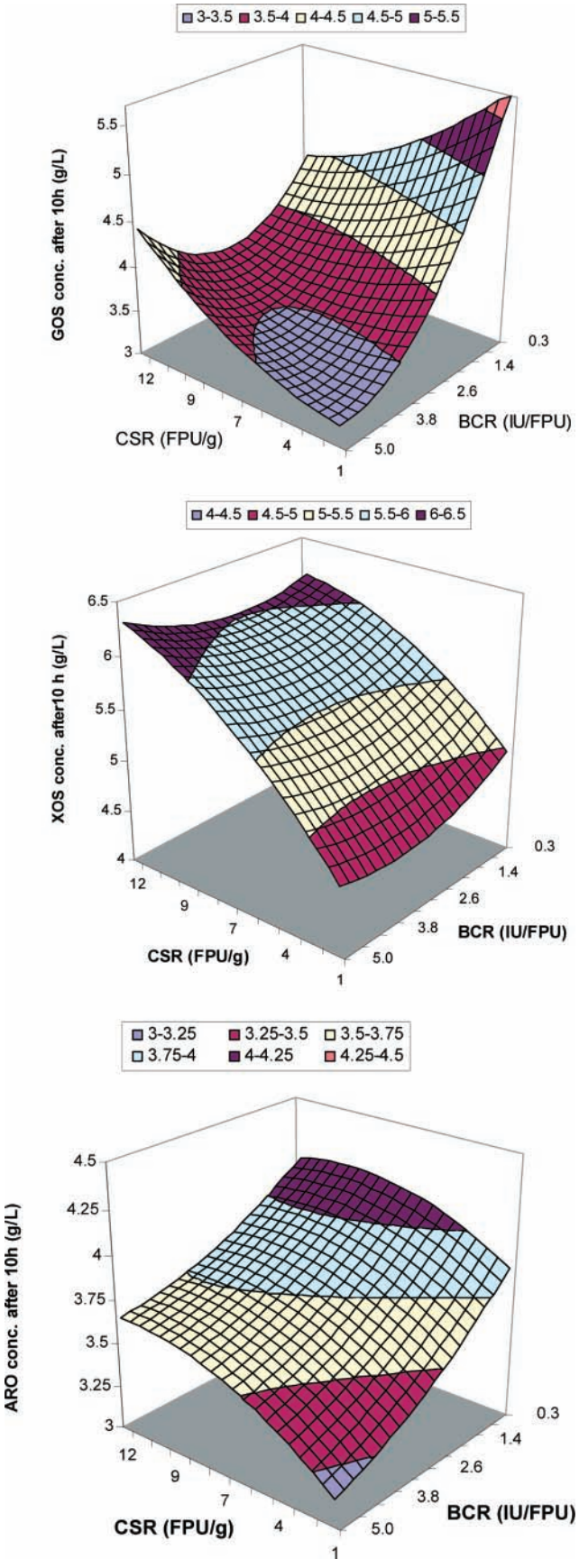

Figure 4. Calculated dependence of (**a**) GOS (glucooligosaccharides), (**b**) XOS (xylooligosaccharides), and (**c**) AOS (arabinooligosaccharides) contents measured at 10 h on the operational variables CSR (cellulase to solid ratio) and BCR (β-glucosidase activity to cellulase activity ratio) for experiments carried out at liquor to solid ratio = 12 g/g.

Table 6. Composition of the Final Fermentation Medium

(**a**) Composition of the Final Liquid Phase

component	content (g/L)
lactic acid	27.8
glucose	0.3
XMnGa	0.38
AR	2.13
GOS	6.01
XOS	4.42
AOS	3.46
uronic acid	6.11
acetic acid	0.3

(**b**) Composition of the Solid Phase

component	content (wt % oven-dry basis)
glucan	21
$XMnGa_n$	10.2
ARn	3.2
acid-insoluble residue	31.6
uronic acids	5.95
others[a]	28.06

[a] Microbial biomass included.

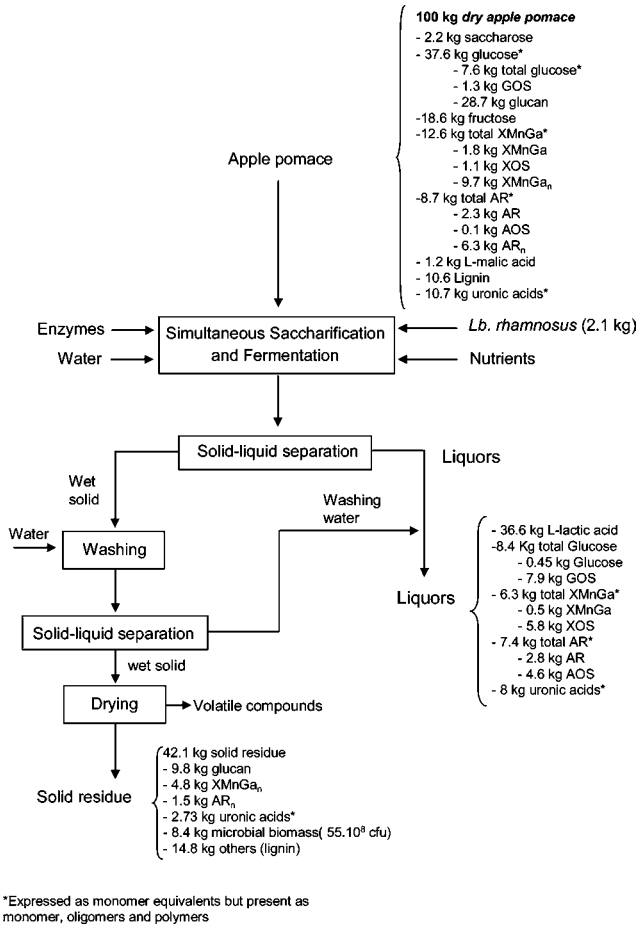

Figure 5. Material balances of the proposed process.

Uronic acids (measured as monomers after total hydrolysis) were not metabolized by bacteria and remained in the medium after SSF (8 kg/100 kg of apple pomace). This fraction is expected to be made up of pectin-derived oligomers suitable for food applications.

Additionally, 18.3 kg of oligosaccharides (GOS, XOS, and AOS) and 8.4 kg of microbial biomass were simultaneously

produced and could contribute to the economic feasibility of the process.

Conclusions. The potential of apple pomace for lactic acid fermentation by SSF was evaluated. The effects of the CSR, the LSR, and the BCR on the kinetics of lactic acid generation were assessed, and a set of mathematical models was developed to reproduce and predict the lactic acid content in fermentation broth.

Operating at low cellulase and cellobiase charges (1 FPU/g and 0.25 IU/FPU, respectively) and short reaction times (10 h), 27.8 g/L of lactic acid was obtained in a fermenter, the volumetric productivity being 2.78 g/Lh. Mass balances showed that 36.6 kg of lactic acid can be produced from 100 kg of dry apple pomace by simultaneous saccharification and fermentation. Moreover, 18.3 kg of oligosaccharides (which can be used as ingredients for functional foods) and 8.4 kg of microbial biomass (which can be used as a probiotic after separation from the undegraded apple pomace) were produced simultaneously. Compounds made up of uronic acids with potential food applications (8 kg/100 kg of apple pomace) were not metabolized by bacteria and remained in the fermentation medium.

ABBREVIATIONS USED

A, regression parameter; ADF, acid-detergent fiber; ADL, acid-detergent lignin; AIF, alcohol-insoluble fraction; AOS, arabinooligosaccharides; BCR, β-glucosidase activity to cellulase activity ratio; CSR, cellulase to solid ratio; E, extractives; FPU, filter paper units; GOS, glucooligosaccharides; IU, International Units; LSR, liquor to solid ratio; MRS, Man, Rogosa, and Sharpe; NDF, neutral-detergent fiber; P_0', initial volumetric productivity to lactic concentration ratio (h^{-1}); P_m, maximum lactic acid concentration (g/L); PNPG, 4-nitrophenyl-β-D-glucopyranoside; SSF, simultaneous saccharification and fermentation; TOS, total oligosaccharides; EU, enzyme units; XOS, sylooligosaccharides.

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