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Simultaneous enzymatic wheat starch saccharification and fermentation to lactic acid by *Lactococcus lactis*

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Abstract Simultaneous saccharification of starch from whole-wheat flour and fermentation to lactic acid (SSF) was investigated. For saccharification the commercial enzyme mixture SAN Super 240 L, having α-amylase, amyloglucosidase and protease activity, was used, and *Lactococcus lactis* ssp. *lactis* ATCC 19435 was used for the fermentation. SSF was studied at flour concentrations corresponding to starch concentrations of 90 g/l and 180 g/l and SAN Super concentrations between 3 µl/g and 8 µl/g starch. Kinetic models, developed for the saccharification and fermentation, respectively, were used for simulation and data from SSF experiments were used for model verification. The model simulated SSF when sufficient amounts of nutrients were available during fermentation. This was achieved with high wheat flour concentrations or with addition of yeast extract or amino acids. Nutrient release was dependent on the level of enzyme activity.

Introduction

Lactic acid is produced either synthetically or by fermentation (Vickroy 1985). Its technical applications include use as a preservative in food, pharmaceuticals and cosmetics, and in the production of poly(lactic acid), a biodegradable polyester used in medical sutures and clips for wound closure, and in self-degradable prosthetic devices (Kharas et al. 1994).

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Cheap raw materials, such as whey, molasses, cellulose, starch waste and beet and cane sugar have been used for the fermentative production of lactic acid by lactic acid bacteria (Vickroy 1985; Parajó et al. 1996). Production of lactic acid from wheat starch (Hofvendahl and Hahn-Hägerdal 1997; Xiaodong et al. 1997; Åkerberg et al. 1998), barley starch (Linko and Javanainen 1996), potato starch (Ray et al. 1991; Xiaodong et al. 1997; Tsai and Millard 1994; Chatterjee et al. 1997), corn starch (Hoshino et al. 1991; Xiaodong et al. 1997; Zhang and Cheryan 1991; Mercier et al. 1992) and wheat bran (Shamala and Sreekantiah 1987) has been reported.

In the current investigation whole-wheat flour was used as a raw material. Wheat flour starch was hydrolysed in two separate steps: liquefaction and saccharification. During liquefaction, starch was solubilised by a thermostable α-amylase, reducing the chain length of the starch. In the subsequent saccharification step, the oligosaccharides were further hydrolysed by the enzyme mixture SAN Super 240 L consisting of an α-amylase and amyloglucosidase, resulting in the production of glucose, and a protease, releasing nutrients. Glucose was fermented to lactic acid by *Lactococcus lactis* ssp. *lactis* ATCC 19435 (*L. lactis* 19435), utilising the nutrients released from the wheat flour. Nutrients in wheat flour (Hofvendahl and Hahn-Hägerdal 1997), wheat bran (Shamala and Sreekantiah 1987), barley (Javanainen and Linko 1995), corn (Childs and Welsby 1966; Mercier et al. 1992) and potato (Ray et al. 1991) are sufficient for the lactic acid bacteria used for lactic acid production.

The saccharification and fermentation steps can be performed separately or simultaneously (SSF). To investigate whether it is advantageous to integrate these two process steps and to optimise this integration, a kinetic model is useful. Previously, models have been developed describing separate saccharification of wheat starch by a mixture of amyloglucosidase and an α-amylase (Åkerberg et al. 1999) and the fermentation of glucose to lactic acid by *L. lactis* 19435 in a whole-wheat

flour medium (Åkerberg et al. 1998). In the present study SSF was investigated at different starch and SAN Super concentrations. Also, the effect of various added nutrients was studied. The models developed for the individual process steps were combined and data from SSF experiments were used to verify the combined model, which was used for simulation of SSF.

Materials and methods

Inoculum preparation and microorganism and enzymes used

The inoculum was prepared in a flour-free, rich medium as previously described (Åkerberg et al. 1998), utilising *Lactococcus lactis* ssp. *lactis* ATCC 19435 (American Type Culture Collection, Rockville, Md., USA). For liquefaction, the thermostable α-amylase Termamyl 120 L (Novo Nordisk, Bagsværd, Denmark; Novo Nordisk 1990) was used. This enzyme has an activity of 120 KNU/g, where 1 KNU is defined as the amount of enzyme needed to break down 5.26 g starch (Merck, Amylum Soluble Erg. B. 6, batch 9947275). In the saccharification the enzyme mixture SAN Super 240 L (Novo Nordisk 1991) was used. This enzyme consists of the α-amylase Fungamyl (1,4-α-D-glucan glucanohydrolase EC 3.2.1.1), amyloglucosidase AMG (1,4-α-D-glucan glucanohydrolase EC 3.2.1.3) and the protease Neutrase 0.5 L. SAN Super 240 L holds an activity of 240 AGU/ml where 1 AGU is defined as the amount of enzyme hydrolysing 1 μmol maltose/min at 25 °C, pH 4.3. To evaluate the effect of omitting protease, only Fungamyl and AMG were added in one fermentation.

Liquefaction

Whole-wheat flour containing gluten and bran, with the most coarse fraction sieved off, was suspended in water, pH 7, to a concentration between 240 g/l and 275 g/l and heated to 50 °C to start the gelatinisation process. Termamyl 120 L was added (200 μl/l) and the mixture was heated to 95 °C and maintained at that temperature for 30 min. Owing to evaporation of water during the liquefaction process, the volume was adjusted with autoclaved water.

Simultaneous saccharification and fermentation

In fermentation of less than 240 g/l wheat flour, the flour suspension was diluted with autoclaved water after liquefaction. SAN Super (3–8 μl/g starch) or Fungamyl and AMG (0.70 and 5.6 μl/g starch respectively) were added with the inoculum. In some fermentation experiments yeast extract (5 g/l), casamino acids (5 g/l) (Difco, Detroit, Micho., USA) and asparagine (0.4 g/l), vitamin solution (10 ml/l) or the tryptone fraction of tri- to heptapeptides (0.5 g/l) were added. The vitamin solution consisted of the following components (mg/l): D-biotin 10, pyridoxal/HCl 206, folic acid 100, riboflavin 100, niacinamide 100, thiamine/HCl 100, Calcium D-pantothenate 95, *p*-aminobenzoic acid 10. Tryptone (2 g) was fractionated on a Sephadex G-25 column (66 × 1.5 cm; flow rate 0.61 ml/min) with 10 mM NH_4HCO_3 as eluent. The fractions (5 ml) were partly pooled, depending on the peak absorption of the eluent at 280 nm. Peptides of three to seven amino acids were added to the fermentation.

SSF was carried out in either a 3-l Chemoferm FLC-B-3 fermentor, as described previously (Åkerberg et al. 1998), or a 3-l Bioflo III fermentor (New Brunswick Scientific, Edison, N.J., USA) with 1 l working volume. Nitrogen was flushed over the culture at 0.1 l/min, controlled by a mass flow controller (Bronkhorst Hi-Tec, Ruurlo, The Netherlands). The pH was controlled at 6 with 250 g/l NaOH (Akzo Nobel, Eka Nobel, Bohus, Sweden), the stirring rate was 100 rpm and the temperature 30 °C.

Analysis

Analysis of the cell mass concentration of the inoculum as dry weight, and of glucose, maltose and product concentrations of the broth using HPLC was performed as described previously (Åkerberg et al. 1998).

Model

Saccharification

A saccharification model has been developed describing the kinetics of enzymatic formation of glucose and conversion of oligosaccharides with a degree of polymerisation (DP) of 2–7 (Åkerberg et al. 1999) by SAN Super, and is presented in Appendix A. The model consists of two parts, one part describing the kinetics of glucose formation and conversion of oligosaccharides by amyloglucosidase action, and the other describing the formation of glucose and oligosaccharides (DP = 2–7) as a result of the action of α-amylase. The model describes the influence of pH, starch and SAN Super concentrations on the saccharification kinetics. The model parameters were determined by fitting to experimental data from saccharification experiments at pH 4–6 with starch concentrations between 0.1 g/l and 150 g/l and SAN Super concentrations between 1 μl/g starch and 7.5 μl/g starch. The parameters were determined at two temperatures, 30 °C and 55 °C, optimal for fermentation and saccharification respectively. The model parameters are summarised in Tables 1 and 2; however, only for pH 6 since this was the only pH used in the present study.

Fermentation

A fermentation model describing the kinetics of cell growth, lactic acid formation and substrate consumption has been developed (Åkerberg et al. 1998) and is shown in Appendix B. The kinetics of acetate formation have been included in the model as a measure of the by-product formation. The model considers the influence of pH, temperature and the inhibition of glucose and lactic acid. The model parameters have been determined by fitting to experimental data from fermentation experiments for both totally hydrolysed wheat flour and unhydrolysed flour supplemented with glucose using *L. lactis* 19435 at pH 4–6, 30–37 °C, and glucose and lactic acid concentrations up to 180 g/l and 89 g/l respectively. Only parameters determined for totally hydrolysed flour at pH 6 and 30 °C were utilised in the present study (Table 3).

During fermentation in unhydrolysed wheat flour medium, small amounts of glucose were produced from unintentional hydrolysis of the starch (Åkerberg et al. 1998). Since no enzymes were added, this glucose production could be due to amylases present in

Table 1 Parameters, dependent on the degree of polymerisation (n = 1–7), for saccharification kinetics at pH 6 (Åkerberg et al. 1999)

n	1	2	3	4	5	6	7
$K_{m,n}$ (g/l)	–	0.157	0.203	0.182	0.167	0.0990	0.139
$k_{max,n}$ (1/h)	–	1.74×10^4	3.59×10^4	6.01×10^4	7.60×10^4	5.76×10^4	7.31×10^4
k_n (30 °C) (1/h)	0.667	0.651	0.229	0.278	0.331	0.0767	0.440
k_n (55 °C) (1/h)	0.600	0.129	0.299	0.324	0.336	0.289	0.724

Table 2 Parameters for saccharification kinetics at pH 6 (Åkerberg et al. 1999)

Parameter	Value
k_{es} (30 °C) (g/l)	1.22×10^{13}
k_{es} (55 °C) (g/l)	6.21×10^{13}
K_g (g/l)	110
k_e (µl AMG/g starch)	1.06×10^{11}
k_s (g/l)	1.77×10^{8}
k_{en} (µl Fungamyl/g starch)	0.309

the flour (Cornell and Hoveling 1998) or to acid hydrolysis. This unintentional hydrolysis was included in the fermentation model. During saccharification with SAN Super the unintentional hydrolysis can be assumed to occur in parallel with the more powerful hydrolysis due to the added SAN Super enzymes, and is thus included in the parameters in the saccharification model. Therefore, in the present study, the unintentional hydrolysis was omitted from the fermentation model. In addition, all SSF experiments were performed at pH 6 and, at this pH, the unintentional hydrolysis of flour was negligible compared with the hydrolysis as result of added enzymes.

Simultaneous fermentation and saccharification

In the present study, the models for saccharification and fermentation were combined to construct the model for SSF. The glucose production rate was described as the sum of the formation due to saccharification (Eq. A6, Appendix A) and consumption due to fermentation (Eq. B4, Appendix B):

$$(r_{G_1})_{SSF} = \left(\frac{dG_1}{dt}\right) + r_{G_1}$$

The saccharification kinetics of the oligosaccharides (DP = 2–7) was described using Eqs. A4–A5 (Appendix A). The kinetics of the cell growth, product and by-product formation was described using Eqs. B1–B3 (Appendix B) respectively.

Results

Simultaneous saccharification and fermentation (SSF) of starch from whole-wheat flour was performed under different physical conditions. Simulations and fermentation experiments showed that the fermentation is the limiting of the two process steps (data not shown)

Table 3 Parameters for fermentation kinetics at 30 °C and pH 6 (Åkerberg et al. 1998)

Parameter	Value
μ_{max} (1/h)	0.532
K_s (g/l)	0.790
K_i (g/l)	164
K_p (l/g)	1.37×10^{-2}
n_P	2.36
α	13.2
β(1/h)	6.45×10^{-2}
α_a	0.332
β_a (1/h)	84.6
Y_x (g/g)	0.79
Y_P (g/g)	1.00
Y_{pa} (g/g)	0.169
m (1/h)	6.78×10^{-3}

and SSF experiments were thus performed at a pH and temperature optimal for *L. lactis* 19435, i.e. pH 6 and 30 °C (Åkerberg et al. 1998). According to the fermentation model (Åkerberg et al. 1998), the cell growth rate is totally inhibited by 73 g/l lactic acid at pH 6. Therefore the majority of the SSF experiments were performed at a low starch concentration (about 90 g/l), resulting in a lactic acid concentration of the same order of magnitude as the inhibition concentration. One experiment was performed at a higher starch concentration (179 g/l). Growth and lactic acid production of *L. lactis* 19435 have been suggested to depend on the release of nutrients from the flour by the activity of SAN Super (Hofvendahl and Hahn-Hägerdal 1997; Åkerberg et al. 1998). SSF experiments were thus performed at different SAN Super concentrations as well as with the addition of nutrients.

The conditions for SSF A-J, the calculated maximum lactic acid formation rates and the lactic acid yields are presented in Table 4. The total starch concentration is expressed as glucose equivalents, i.e. the amount of glucose formed from totally hydrolysed starch. The higher starch concentration doubled the maximum lactic acid formation rate, compared to when a lower starch concentration was used. At the lower starch concentration, addition of nutrients increased the formation rate by 50%–90% (Table 4). When the protease was omitted during SSF at the lower starch concentration, the formation rate decreased significantly. For SSF experiments at the lower starch concentrations without yeast extract addition, the highest maximum lactic acid formation rate was achieved at a SAN Super concentration of 6–8 µl/g starch. The lactic acid yield was high (at least 90%) for all SSF experiments (Table 4) except when protease was omitted.

Simulation of all SSF experiments was performed using initial concentrations of glucose, oligosaccharides DP = 2–7, starch, cell mass, lactic acid and acetic acid (Table 5) and parameter values determined in previous studies (Tables 1–3). The initial concentrations of glucose, maltose, lactic and acetic acid were experimentally determined. Because of the high particle content in wheat flour, cell mass could not be measured during fermentation and the initial cell mass concentration was measured in the flour-free inoculum. The initial concentrations of oligosaccharides larger than maltose were estimated from earlier saccharification experiments (Åkerberg et al. 1999). Experimental data of glucose, maltose and lactic acid concentrations for SSF A–C are shown together with simulated data in Fig. 1. The model predicted the kinetics of the saccharification and fermentation at a high starch concentration, SSF A (Fig. 1A). At a lower starch concentration without nutrient addition, SSF B, G–J, the lactic acid formation rate was lower than predicted by the model (Fig. 1B). However, when nutrients, in the form of yeast extract or amino acids, were added (SSF C, D), the lactic acid formation rate increased and the model simulated SSF well (Fig. 1C). Addition of vitamins and peptides (SSF

Table 4 Experimental conditions, lactic acid formation rates and lactic acid yields of SSF experiments

SSF	Total starch concentration (g/l)[a]	Nutrient addition[b]	Enzyme[c] concentration (μl/g starch)	AMG concentration[d] (μl/g starch)	Fungamyl concentration[d] (μl/g starch)	Maximum lactic acid formation rate (g/l · h)	Lactic acid yield[e] (%)
A	179	–	6.8	5.4	0.68	3.2	99
B	92	–	6.7	5.4	0.67	1.5	100
C	91	yeast extract	6.6	4.7	0.66	3.3	100
D	89	amino acids	6.7	4.8	0.67	2.8	90
E	88	vitamins	6.8	4.9	0.68	2.4	91
F	89	peptides	6.7	5.4	0.67	2.2	90
G	96		6.3[f]	5.6	0.70	0.23	77
H	96	–	3.1	2.5	0.31	1.2	95
I	90	–	5.0	4.0	0.50	0.85	92
J	94	–	8.1	6.5	0.81	1.7	97

[a] Total starch concentration expressed as glucose equivalents
[b] For concentrations, see Materials and methods
[c] SAN Super when not otherwise indicated
[d] Assuming that SAN Super contains 80% AMG and 10% Fungamyl
[e] Yield calculated as the amount of lactic acid formed relative to the amount of starch consumed at the end of the SSF experiment $(P_{t1} - P_{t0})/(S_{0,t1} - S_{0,t0})$, where t_0 represents the end of the lag phase and t_1 the time when the product concentration has reached the product inhibition concentration, 73 g/l, or the last time point in the SSF experiment
[f] Fungamyl and AMG, see Materials and methods

E, F) did not increase the lactic acid formation rate sufficiently to achieve a good fit between experimental data and the model.

To compare the two process alternatives, separate and simultaneous saccharification and fermentation for the low wheat starch concentration, provided that sufficient nutrients were available, was simulated (Fig. 2). For the simulation of the saccharification step alone, kinetic parameters determined at 55 °C (Tables 1 and 2) were used, since this is the optimal temperature for the enzyme mixture used for saccharification. However, for SSF, kinetic parameters determined at 30 °C were used. The SSF required a lower total residence time than did the separate saccharification and fermentation, and glucose was totally consumed after 104 h and 112 h for the SSF and the separate saccharification and fermentation respectively. The maximum lactic acid formation rate was 3.6 g l^{-1} h^{-1} for both the SSF and the separate saccharification and fermentation.

SSF was also simulated to investigate the influence of the enzyme concentration on the lactic acid production rate. Simulation showed that the SAN Super concentration could be reduced to 1 μl/g starch without the saccharification being rate-limiting during SSF.

Discussion

The developed model simulated SSF when the wheat flour concentration was high and when yeast extract or amino acids were added to a fermentation of low wheat flour concentration (Fig. 1A, C). The lactic acid formation rate was lower than predicted by the model for SSF experiments using low wheat flour concentration without nutrient addition or with vitamins or peptide addition (SSF B, C, E–H) (Fig. 1B). This indicated that either the saccharification model or the fermentation model was not able to describe the SSF at a low wheat flour concentration. Neither the model nor the experimental data showed glucose limitation until the end of SSF. Thus, the fermentation step was rate-limiting, and the fermentation model was unable to simulate SSF at a low wheat flour concentration. This could be due to nutrient limitation for *L. lactis* 19435 during SSF.

Previous studies (Hofvendahl and Hahn-Hägerdal 1997; Åkerberg et al. 1998) have indicated that enzymatic hydrolysis of wheat flour using the SAN Super mixture released necessary nutrients for growth and lactic acid production of *L. lactis* 19435. Wheat flour contains proteins, vitamins B and E, lipids and minerals

Table 5 Initial concentrations of simulated components. The initial lactic acid and acetic acid concentrations were 0 g/l in all SSF experiments. G_{SLarge} is the total concentration of non-soluble polysaccharides and soluble oligosaccharides with more than 7 glucose units

SSF	Component concentration (g/l)								Cell mass (g)
	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_{SLarge}	
A	66	49	25	3.1	1.7	1.8	0.49	27	0.011
B	34	35	7.5	1.2	0.64	0.69	0.19	10	0.011
C	41	27	7.4	1.3	0.66	0.71	0.20	11	0.022

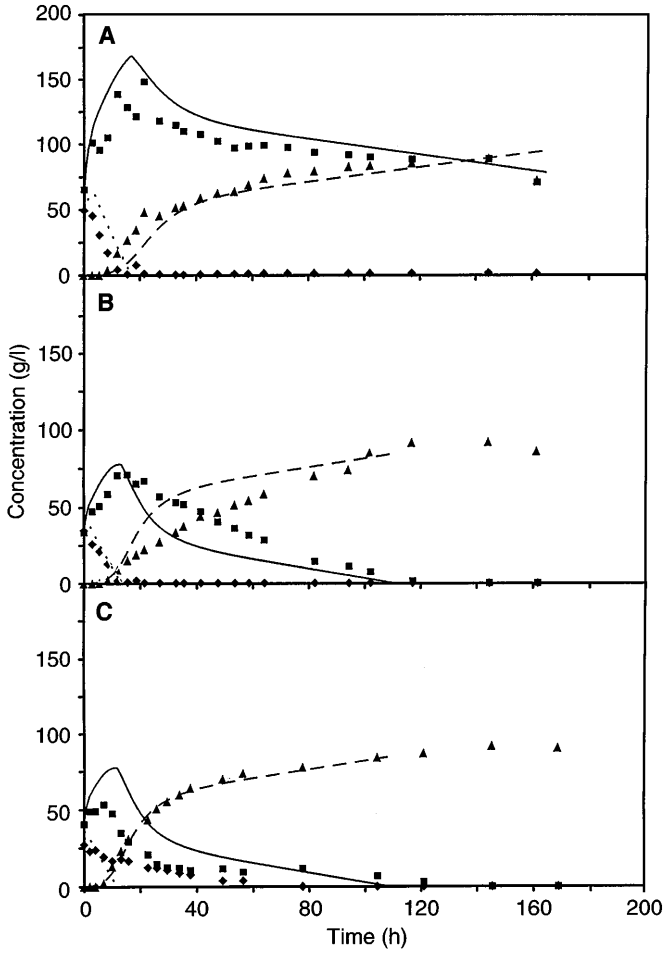

Fig. 1A–C Simulation and experimental data for simultaneous saccharification and fermentations **A–C** at a high starch concentration (**A**), low starch concentration without nutrient addition (**B**) and low starch concentration with yeast extract addition (**C**). Experimental conditions as in Table 4. Experimental data and simulated results for glucose (■ ——), lactic acid (▲, – – –) and maltose (◆, - - -)

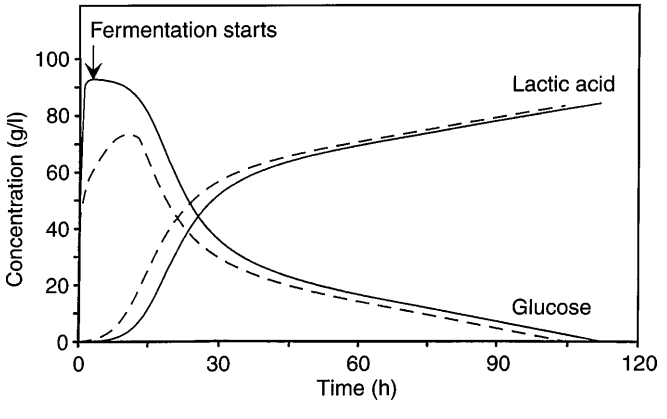

Fig. 2 Simulation of glucose and lactic acid concentrations at low wheat starch concentration (90 g/l) in a system provided with sufficient nutrients and a SAN Super concentration of 6 µl/g starch for the saccharification and fermentation performed as two separate steps (———) and simultaneously (– – –)

in addition to carbohydrates (Cornell and Hoveling 1998). Omission of the protease from SAN Super reduced the lactic acid formation rate substantially. The protease in SAN Super produces peptides and/or amino acids from flour proteins, and *Lactococci* peptidases hydrolyse the peptides to amino acids (Pritchard and Coolbear 1993). In fermentation of ragi or wheat bran by *Lactobacillus delbrueckii*, addition of α-amylase, amyloglucosidase and a protease increased the amino acid content and the yield of lactic acid from 36% to 90% (Shamala and Sreekantiah 1987). The addition of yeast extract or amino acids increased the lactic acid formation rate whereas addition of vitamins and peptides, at the concentrations studied, had an intermediary effect on the lactic acid production rate.

The fermentation model used in the present investigation is based upon fermentation experiments using a medium of totally hydrolysed wheat flour at both low and high concentrations, and does not consider nutrient limitation (Åkerberg et al. 1998). This medium was saccharified at a temperature and pH optimal for SAN Super (55 °C and pH 5), so that all nutrients had been released prior to the fermentation. However, in SSF, nutrients were continuously released during fermentation as a result of SAN Super activity, and at suboptimal conditions for SAN Super (30 °C and pH 6). At high wheat flour concentration, SSF A, the levels of nutrients per cell were higher. This wheat flour concentration has been shown to provide sufficient nutrients, and the lactic acid formation rate was not affected by yeast extract addition (Hofvendahl and Hahn-Hägerdal 1997). Nutrient limitation is likely to have occurred at the high wheat flour concentration as well, but not until the fermentation had reached the product inhibition region, which masked the nutrient limitation. However, at a low wheat flour concentration, the nutrient limitation occurred before the product inhibition, and lower product formation rate due to nutrient limitation was observed.

When the SAN Super concentration was increased from 5 µl/g to 6 µl/g starch in the SSF experiments at a low wheat flour concentration (SSF B and E), the maximum lactic acid formation rate increased by 194% (Table 4). In addition, when protease was omitted the lactic acid formation rate decreased significantly. This indicates that the release of nutrients is dependent both on the enzyme concentration and composition.

The simultaneous saccharification and fermentation was simulated to compare separate and simultaneous saccharification and fermentation. Fermentation at high wheat flour concentrations is strongly product inhibited (Åkerberg et al. 1998), and hence it is appropriate to use a lower wheat flour concentration in an industrial process. In addition, results from the present study showed that nutrient addition significantly improves the lactic acid production rate in SSF. Consequently, the simulations were performed for low wheat flour concentrations assuming 6 µl SAN Super/g starch, and sufficient nutrients to maintain growth and lactic acid production of *L. lactis* 19435 at a maximum level. This requires extra

addition of amino acids, e.g. yeast extract. Simulation showed that SSF was somewhat faster than separate saccharification and fermentation (Fig. 2). In SSF, inhibition by glucose, as a product in saccharification and a substrate in fermentation, is less than when the two process steps are performed separately. However, since both these inhibition effects are relatively small (Åkerberg et al. 1998, 1999), the difference in residence time was also small, and the maximum lactic acid formation rate was the same for the two process alternatives. SSF of cellulose to lactic acid by *Lactobacillus delbrueckii* has also been shown to give higher yield and productivity than if the two steps are performed separately (Abe and Takagi 1991).

SSF at a low wheat flour concentration was simulated to investigate the influence of the enzyme concentration on the lactic acid production rate. It was observed that the enzyme concentration could be decreased from 6 μl/g to 1 μl/g starch without the saccharification being rate-limiting, provided that sufficient nutrients were available. Thus, for enzyme concentrations higher than 1 μl/g starch the fermentation is rate-limiting. This implies that optimum conditions for SSF are the same as for the fermentation, i.e. 30 °C and pH 6. For SSF of cellulose to lactic acid by *Lactobacillus bulgaricus* the fermentation is rate-limiting below pH 5 (Venkatesh 1997).

Simulation showed that production of lactic acid with SSF decreases the requirement for enzymes, thus decreasing the cost. However SSF with wheat particles present could cause problems in the downstream processing if the bacteria were to be recycled back to the fermentor. By separating the saccharification and fermentation steps, the saccharification can be performed at optimum temperature and pH, thus increasing the productivity. In addition, the fermentation can be performed without addition of extra nutrients. However, separate saccharification and fermentation requires extra equipment for control and adjustment as well as higher SAN Super concentration to reduce the residence time in the saccharification step. Downstream processing, on the other hand, is simplified since the wheat flour can be removed prior to fermentation. Downstream processing and the economics of enzymes and nutrients addition must be further studied and modelled to determine optimal operating conditions for the total process.

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Appendix A. Saccharification model (Åkerberg et al. 1999)

The cleavage rate of an oligosaccharide with n glucose units due to the action of the amyloglucosidase AMG was described as (g/l · h):

$$v_{G_n} = -\frac{V_{\max,n} \cdot G_n}{K_{m,n} + G_n} \cdot \frac{K_g}{K_g + G_1} \qquad (n = 2 \text{ to } 7) \tag{A1}$$

where G_n is the concentration of glucose or oligosaccharides with n glucose units (g/l) (adjusted for NaOH addition), K_g is the glucose inhibition parameter (g/l) and $K_{m,n}$ is the Michaelis-Menten constant for an n-mer oligosaccharide (g/l). The maximum reaction rate for an n-mer oligosaccharide (g/l · h), $V_{\max,n}$ was described by:

$$V_{\max,n}(E, S_0) = k_{\max,n} \cdot k_{es} \cdot \frac{E_{AMG}}{E_{AMG} + k_e} \cdot \frac{S_0}{S_0 + k_s} \qquad (n = 2 \text{ to } 7) \tag{A2}$$

where $k_{\max,n}$ is the maximal velocity for digestion rate of an n-mer oligosaccharide (1/h), k_{es} a rate parameter (g/l), k_e the amyloglucosidase limitation parameter (μl AMG/g starch), k_s the starch limitation parameter (g/l), E_{AMG} the amyloglucosidase concentration (μl AMG/g starch) and S_0 the total starch concentration, expressed as glucose equivalents (g/l).

The total formation rate of glucose and oligosaccharides with a degree of polymerisation from 2–7 in a batch reactor was described as the sum of the net production rate due to the action of amyloglucosidase and the formation rate from oligosaccharides with a degree of polymerisation larger than 7 (G_{SLarge}) due to the action of the α-amylase Fungamyl:

$$\frac{dG_{SLarge}}{dt} = -\left(\sum_{n=1}^{7} k_{SLarge,n}\right) \cdot G_{SLarge} \tag{A3}$$

$$\left(\frac{dG_7}{dt}\right) = v_{G_7} + k_{SLarge,7} \cdot G_{SLarge} \tag{A4}$$

$$\left(\frac{dG_n}{dt}\right) = v_{G_n} - v_{G_{n+1}} \cdot \frac{MW_n}{MW_{n+1}} + k_{SLarge,n} \cdot G_{SLarge} \qquad (n = 2 \text{ to } 6) \tag{A5}$$

$$\left(\frac{dG_1}{dt}\right) = -\left(\sum_{j=3}^{7} v_{Gj} \cdot \frac{MW_1}{MW_j}\right) - 2 \cdot v_{G_2} \cdot \frac{MW_1}{MW_2} + k_{SLarge,1} \cdot G_{SLarge} \tag{A6}$$

where the terms V_{G_n} describe the net production rate due to the action of amyloglucosidase and $k_{SLarge,n} \cdot G_{SLarge}$ describe the production rate of an n-mer oligosaccharide due to the action of the α-amylase. MW_n is the molecular weight of glucose or oligosaccharides containing n glucose units (g/mole)

$$k_{SLarge,n} = k_n \cdot \frac{E_\alpha}{k_{en} + E_\alpha} \qquad (n = 1 \text{ to } 7) \tag{A7}$$

where k_n is a parameter for the formation of glucose of an n-mer oligosaccharide from larger oligosaccharides (1/h), k_{en} the α-amylase limitation parameter (μl Fungamyl/g starch) and E_α is the α-amylase concentration (μl Fungamyl/g starch).

Appendix B. Fermentation model (Åkerberg et al. 1998a)

$$\text{Cell growth rate } r_x = \frac{\mu_{max} \cdot G_1 \cdot X}{G_1 + K_s + \frac{(G_1^*)^2}{K_i}} \left(1 - K_p \cdot P^*\right)^{n_P} \quad \text{(B1)}$$

X, G_1 and P are the cell mass, substrate and the product concentration (g/l) respectively, *concentrations not adjusted for NaOH addition, μ_{max} is the maximum specific growth rate (1/h), K_s the saturation parameter (g/l), K_i the glucose inhibition parameter (g/l) and K_p and n_P are parameters describing lactic acid inhibition.

$$\text{Lactic acid formation rate } r_p = \alpha \cdot r_x + \beta \cdot X \quad \text{(B2)}$$

α and β are the growth- and non-growth-associated constants respectively for lactic acid production.

$$\text{Acetic acid formation rate } r_{pa} = \alpha_a \cdot r_x + \beta_a X \quad \text{(B3)}$$

$\alpha_A$and β_A are the growth- and non-growth-associated constants respectively for the acetic acid production.

$$\text{Glucose consumption rate } r_{G1} = -\frac{1}{Y_p} \cdot r_p - \frac{1}{Y_{pa}} \cdot r_{pa} - \frac{1}{Y_x} \cdot r_x - m \cdot X \quad \text{(B4)}$$

Y_p (g lactic acid/g glucose) and Y_x (g cell mass/g glucose) are the stoichiometric yield coefficients for lactic acid and cell mass production from glucose. Y_{pa} (g by-products/g glucose) is the yield factor for by-product formation from glucose and m is a parameter for maintenance energy (1/h).

References

Abe S-I, Takagi M (1991) Simultaneous saccharification and fermentation of cellulose to lactic acid. Biotechnol Bioeng 37: 93–96

Åkerberg C, Hofvendahl K, Zacchi G, Hahn-Hägerdal B (1998) Modelling the influence of pH, temperature, glucose and lactic acid concentrations on the kinetics of lactic acid production by *Lactococcus lactis* ssp. *lactis* ATCC 19435 in whole-wheat flour. Appl Microbiol Biotechnol 49: 682–690

Åkerberg C, Zacchi G, Torto N, Gorton L (1999) A kinetic model for the enzymatic saccharification of wheat starch. Submitted

Chatterjee M, Chakrabarty SL, Chattopadhyay BD, Mandal RK (1997) Production of lactic acid by direct fermentation of starchy wastes by an amylase-producing *Lactobacillus*. Biotechnol Lett 19: 873–874

Childs CG, Welsby B (1966) Continuous lactic fermentation. Process Biochem 1: 441–444

Cornell HJ, Hoveling AW (1998) Wheat – chemistry and utilization. Technomic, Pennsylvania, USA

Hofvendahl K, Hahn-Hägerdal B (1997) L-Lactic acid production from whole-wheat starch hydrolysate using strains of *Lactobacilli* and *Lactococci*. Enzyme Microb Technol 20: 301–307

Hoshino K, Taniguchi M, Maumoto H, Shimizu K, Fujii M (1991) Continuous lactic acid production from raw starch in a fermentation system using a reversibly soluble-autoprecipitating amylase and immobilized cells of *Lactobacillus casei*. Agric Biol Chem 55: 479–485

Javanainen P, Linko Y-Y (1995) Lactic acid fermentation on barley flour without additional nutrients. Biotechnol Tech 9: 543–548

Kharas GB, Sanchez-Riera F, Severson DK (1994) Polymers of lactic acid. In: Mobley DP (ed) Plastics from microbes: microbial synthesis of polymers and polymer precursors. Hanser, Munich, pp 93–137

Linko Y-Y, Javanainen P (1996) Simultaneous liquefaction, saccharification, and lactic acid fermentation on barley starch. Enzyme Microb Technol 19: 118–123

Mercier P, Yerushalmi L, Rouleau D, Dochain D (1992) Kinetics of lactic acid fermentation on glucose and corn by *Lactobacillus amylophilus*. J Chem Technol Biotechnol 55: 111–121

Novo Nordisk (1990) Product sheet for Termamyl B552a-GB 1500. Novo Nordisk, Bagsvaerd

Novo Nordisk (1991) Product sheet for SAN Super B416c-GB2000. Novo Nordisk, Bagsvaerd

Parajó JC, Alonso JL, Santos V (1996) Lactic acid from wood. Process Biochem 31: 271–280

Pritchard GG, Coolbear T (1993) The physiology and biochemistry of the proteolysis in lactic acid bacteria. FEMS Microbiol Rev 12: 179–206

Ray L, Mukherjee G, Majumdar SK (1991) Production of lactic acid from potato fermentation. Indian J Exp Biol 29: 681–685

Shamala TR Sreekantiah KR (1987) Degradation of starchy substrates by a crude enzyme preparation and utilization of the hydrolysates for lactic fermentation. Enzyme Microb Technol 9: 726–729

Tsai TS, Millard CS (1994) Improved pretreatment process for lactic acid production. PCT Patent WO 94/13826

Venkatesh KV (1997) Simultaneous saccharification and fermentation of cellulose to lactic acid. Biores Technol 62: 91–98

Vickroy TB (1985) Lactic acid. In: Blanch HW, Drew S, Wang DIC (eds) The practice of biotechnology: commodity products, vol 3, Pergamon, Elmsford, NY, pp 761–776

Xiaodong W, Xuan G, Rakshit SK (1997) Direct fermentative production of lactic acid on cassava and other starch substrates. Biotechnol Lett 19: 841–843

Zhang DX, Cheryan M (1991) Direct fermentation of starch to lactic acid by *Lactobacillus amylovorus*. Biotechnol Lett 13: 733–738