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Optimization of an Innovative Hollow-Fiber Process to Produce Lactose-Reduced Skim Milk

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Abstract

The research field for applications of lactose hydrolysis has been investigated for several decades. Lactose intolerance, improvement for technical processing of solutions containing lactose, and utilization of lactose in whey are the main topics for development of biotechnological processes. We report here the optimization of a hollow-fiber membrane reactor process for enzymatic lactose hydrolysis. Lactase was circulated abluminally during luminal flow of skim milk. The main problem, the growth of microorganisms in the enzyme solution, was minimized by sterile filtration, ultraviolet irradiation, and temperature adjustment. Based on previous experiments at 23 ± 2°C, further characterization was carried out at 8 ± 2°C, 15 ± 2°C (β-galactosidase), and 58 ± 2°C (thermostable β-glycosidase) varying enzyme activity and flow rates. For a cost-effective process, the parameters 15 ± 2°C, 240 U/mL of β-galactosidase, an enzyme solution flow rate of 25 L/h, and a skim milk flow rate of about 9 L/h should be used in order to achieve an aimed productivity of 360 g/(L·h) and to run at conditions for the highest process long-term stability.

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Index Entries: Lactose hydrolysis; hollow-fiber module; β-galactosidase; thermostable; *CelB* β-glycosidase; diffusional reactor.

Introduction

Every year, 3.2 million t of lactose, dissolved in whey, is accrued by cheese production worldwide *(1)*. Almost half of this amount is used for human and animal nutrition. The rest is waste, which is difficult to dispose of and adds to environmental pollution. Therefore, the need for investigating further utilization possibilities of whey-derived lactose is obvious. One of these applications with a high technological and dietetic interest is the enzymatic hydrolysis of lactose, whose economic importance has been increasing since the 1960s.

In addition to the medical aspect of lactose intolerance, some very important technological advantages result from the lactose hydrolysis into glucose and galactose. For example, solubility increases from 18 to 55% (w/v) at 80% conversion, and sweetness rises to 70% related to sucrose. Furthermore, there is a decrease in the freezing point, an increase in the probability of nonenzymatic browning reactions, and a faster fermentation process in lactose-hydrolyzed medium. Thus, the production of self-sweetening products or products with less sucrose addition would be possible by using lactose-hydrolyzed milk or whey. In addition, positive effects on the crystallization and process properties would be achieved after lactose hydrolysis *(2)*.

Several technologies for enzymatic hydrolysis of lactose exist *(3–5)*. The easiest method is the discontinuous batch process. After reaching the aimed conversion, the reaction is stopped by heating, which causes enzyme denaturation and, consequently, the loss of enzymatic activity. Furthermore, the enzymes become product components after the process.

Immobilization can be employed to use the enzyme's activity for as long as possible. It can be affected by physical or chemical binding on a solid matrix such as glass surfaces, cellulose acetate, or oxiran-gel *(6)*. The high cost of the immobilizing steps, activity loss during immobilization, and the occurrence of hygienic problems because of the fat and protein content in milk and whey *(7)* have a detrimental effect on the decision to choose this process.

A third possibility for enzymatic hydrolysis of lactose is "physical immobilization" by separating the enzyme solution from the substrate flow via ultrafiltration membranes *(8)*. This system causes workable and cheap enzyme fixation with little loss of the catalytic activity of the enzyme. Further advantages of this process are the continuous operation of the reactor at low pressure and the selectivity control by selection of suitable membranes. Application of a high enzyme concentration, easy replacement and regeneration of spent enzyme solution, and little loss of enzymes owing to washout are possible. A drawback is that the diffusion resistance of the hollow-fiber membrane seems to be the limiting factor at high conversion rates.

The economic aim was to develop a hollow-fiber process that is at least as cheap as the batch process. To reach cost-effectiveness, it was calculated that 75% lactose hydrolysis in skim milk at a flow rate of 10 L/h (productivity of 360 g/[L·h]) through one hollow-fiber cartridge should be achieved. The development and production of ultrafiltration modules as steam-sterilizable plastic membranes *(9–11)* are too expensive to reach the economic aim. Thus, it was necessary to find another solution to control the microbiological growth in the system. Our research group *(12)* established the use of a combination of sterile filtration and ultraviolet (UV) irradiation in order to overcome this problem. By this method, it was possible to stabilize the enzyme solution, to produce continuously at a temperature of 23 ± 2°C, and to achieve the aimed productivity. However, long-term stability must be proven. To optimize this process, the influence of temperature was studied. Lower temperature means lower enzyme activity of the used β-galactosidase; lower diffusion rates; and, consequently, lower lactose conversion rates, but it also leads to decreased microbiological stress of the enzyme solution. Therefore, process characterization at different temperatures was carried out to determine the best compromise among process productivity, stabilization, and costs. Additionally, the application of a β-glycosidase (CelB) was tested at 58 ± 2°C in order to prove the possibility of hydrolyzing lactose directly in skim milk and pasteurizing at the same time.

Material and Methods

Functional Principles of the Bioreactor System

An ultrafiltration module consists of a bundle of small, hollow-fibers. The outside (shell side) compartment is constructed as a closed circulation and is filled with enzyme solution. A continuous flow of the substrate (skim milk) is applied to the inside (tube side) of the hollow-fiber membranes. The spatial enzyme separation from the substrate solution is guaranteed by the selected membrane cutoff value (Fig. 1). In this way, continuous, enzymatic lactose hydrolysis is possible without inherent problems of immobilized enzymes. The driving force for this system is the lactose diffusion, which mainly depends on the concentration gradient, the temperature, and the flow rates of the substrate and the enzyme solution.

Figure 2 shows the standard flow sheet of the newly developed plant. The reactor is a hollow-fiber module (43-in module, 10-kDa cutoff, 4.9-m^2 membrane area) consisting of a bundle of capillaries made of polysulfone membranes. The shell-side volume is about 2.5 L and the tube-side volume is about 0.65 L. Skim milk is pumped through the hollow-fiber module. Before enzymatic conversion takes place in the hollow-fiber module, skim milk passes a heat exchanger, a manometer, and a thermometer. After the exit of the module, the lactose-hydrolyzed product can be collected. The enzyme solution is pumped in a closed circulation. The temperature of the enzyme solution was adjusted with a water bath located before the

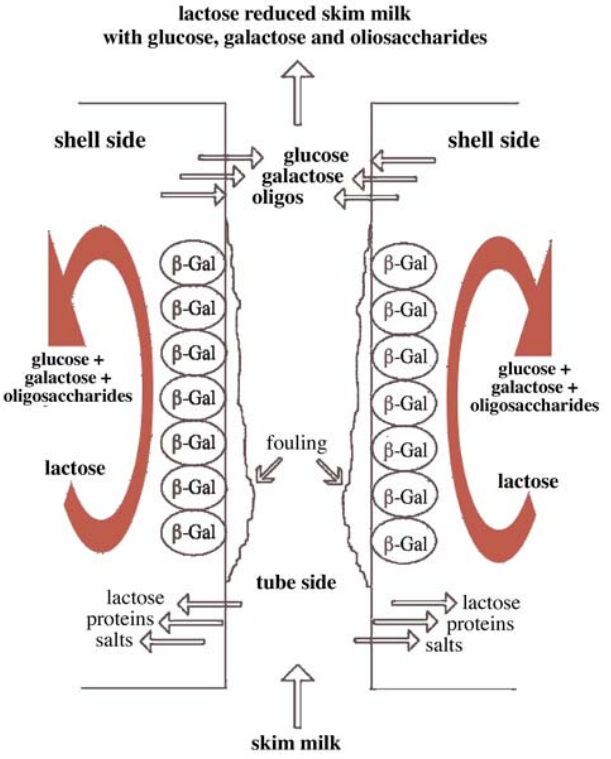

Fig. 1. Principle of lactose hydrolysis in hollow-fiber module by β-galactosidase, denoted as β-gal. While skim milk is pumped through the hollow fiber, molecules with a lower size than the membrane cutoff value such as small proteins, salts, and lactose pass through into the shell side. There, lactose is enzymatically converted into glucose, galactose, and a small amount of oligosaccharides by β-galactosidase. The product transfer back into the tube side is also affected by concentration gradient-driven diffusion.

hollow-fiber's shell side. The hollow-fiber module and the tubing system were covered with a 2-mm insulation felt obtained from Prenner (Austria). For experiments at 8±2°C, an F40 cooling bath from Julabo was used with 1:1 water/ethylene glycol as coolant, and for studies at 15±2°C, tap water was applied for cooling. Tests at 45± 2°C were accomplished with Thermostat Typ K2 heating baths from Lauda. For experiments at 58±2°C with thermostable β-glycosidase, two heat exchangers were essential to achieve sufficient heating of skim milk. They were connected in a row, and skim milk was pumped through the outer part. Skim milk was heated by hot water circulating inside the first loop and outside through a double shell in the second heat exchanger. Additionally, tubing from the exit of the second heat exchanger covered the outside of the first heat exchanger before it was connected to the water bath. Using this assembly, constant heat distribution and sufficient heating were achieved. The heat-exchange row was adjusted in a 30° position to the vertical hollow-fiber module. The surface of the enzyme-heating bath was sealed with a clingwrap.

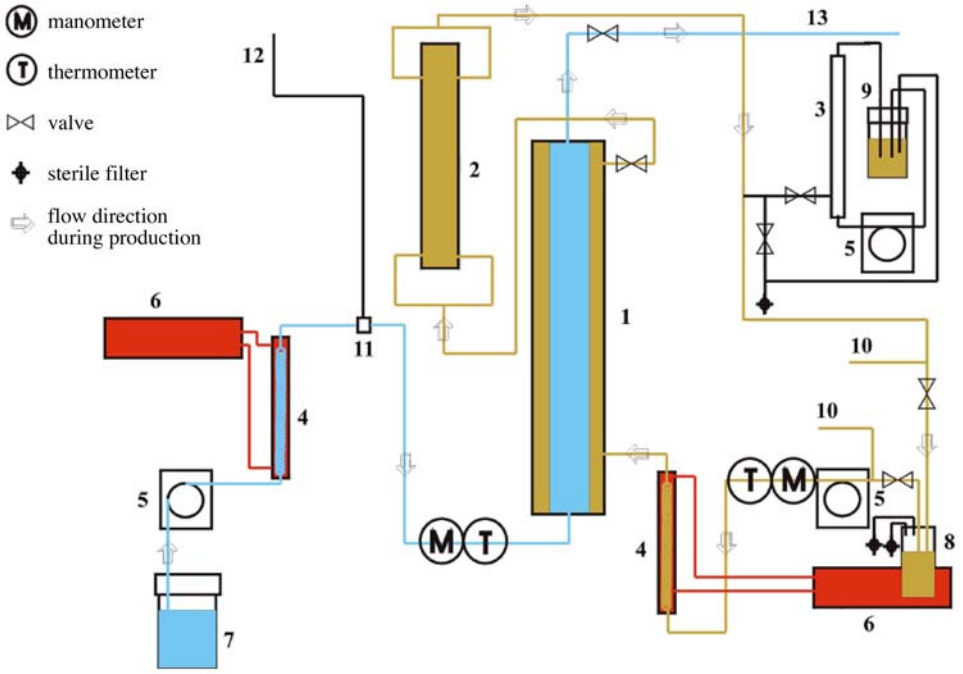

Fig. 2. Scheme of hollow-fiber reactor for lactose hydrolysis by β-galactosidase: 1, hollow-fiber module; 2, UV module; 3, sterile filtration module; 4, heat exchanger; 5, peristaltic pump; 6, heating bath; 7, storage tank for substrate; 8, tank for enzyme solution; 9, tank for sterile filtration circulation; 10, sampling port; 11, three-way cock; 12, waste line; 13, product collection.

A UV irradiation module (Visa™; Elektro Sachs, Austria) and a sterile filtration unit were included in the enzyme circulation in order to sufficiently reduce the germ number. Unless otherwise specified, continuous sterile filtration and 10 min/h UV irradiation at 45% intensity were performed. During experiments with 0.02% (w/v) NaN_3 added to skim milk, neither UV irradiation nor sterile filtration was applied.

Before adding enzyme solution into the enzyme tank, the membrane surface was covered with proteins in order to minimize enzyme loss owing to adsorption. Therefore, skim milk was pumped luminally and was ultrafiltered through the hollow-fiber membranes into the shell side (enzyme circuit). This was followed by circulation of this whey in the shell side for 1 h. Then the enzyme tank was filled with enzyme solution, and lactose hydrolysis experiments started. At the beginning of every study, a steady state of the diffusing substances had to be set between the enzyme solution and the skim milk. After steady state was reached, constant measure values and production were possible. Lactose, glucose, galactose, and enzyme activity were determined from samples taken at several time points. For studies of enzyme concentration-dependent lactose conversion, the enzyme concentration was varied at constant flow rates. Enzyme activity was adjusted by dosages to the enzyme tank.

Apparatus

The hollow-fiber module RomiPro 5 in. × 43 in. PM 10 was from Koch, sterile filters FG-50 and FG-30 were purchased from Millipore (Bedford, MA), and sterile filtration module type MD 020 GP 2N was obtained from Microdyn (Germany).

Chemicals

o-Nitrophenyl-β-D-galactopyranoside (ONPG), *o*-nitrophenol (ONP), and D-glucose were purchased from Sigma (St. Louis, MO); and lactose was from Merck. For skim milk, "medium heat extra skim milk powder" was dissolved in bidistilled water. The powder was produced by Lactoprot and consisted of the following components: 35% protein, 1.25% fat, 51% lactose, 8% ashes, and less than 4% water.

β-*Galactosidase*

The systematic name for β-galactosidase is β-D-galactoside-galactohydrolase (EC 3.2.1.23). Maxilact L2000 and Maxilact LX 5000 from *Kluyveromyces lactis* were obtained from Gist-brocades (The Netherlands). They catalyze lactose hydrolysis.

β-*Glycosidase*

The gene encoding β-glycosidase (*CelB*) from *Pyrococcus furiosus* (DSM 3638) was overexpressed in *Escherichia coli* BL21 (DE3) by using the vector pLUW511; the procedure was similar to that described by Voorhorst et al. *(13)*. Bioreactor cultivations of *E. coli* in the presence of the inductor isopropyl-1-thio-β-D-galactoside (0.1 m*M*) gave approx 100,000 U of enzyme activity/L of culture medium after 8 h of growth *(14)*. Purification was done by a single-step heat treatment (80°C, 30 min) for thermoprecipitation of mesophilic bacterial protein. Petzelbauer et al. *(15)* reported the specificity of β-glycosidase for lactose hydrolysis. The enzyme was supplied by Dr. J. van der Oost (Wagingen, The Netherlands).

Analysis

Determination of Enzyme Activity

To determine the enzyme activity of thermostable β-glycosidase, the method described by Petzelbauer et al. *(15)* was used with slight modification for β-galactosidase Maxilact L2000 and LX 5000 *(12)*. ONPG was utilized as a substrate, and enzyme activity was measured at a constant concentration of ONPG. The release of ONP caused an increase in the absorbance at 405 nm, which was measured for 5 min at 25°C. One unit of ONPG activity is defined as 1 μmol of released ONP/min under the reaction conditions just described.

Sugar Analysis

Quantitative glucose determination was accomplished with an Ebio glucose analyzer from Eppendorf. The measurement range is between 0.5 and 50 m*M* glucose. Consequently, the milk samples were diluted with bidistilled water in order to achieve suitable concentrations. Sample preparation and analysis have been explained previously *(12)*.

High-performance liquid chromatography (HPLC) was used to quantify lactose, galactose, and glucose concentrations. Samples were precipitated by Carrez clarification with 85 m*M* $K_4[Fe(CN)_6]$ and 250 m*M* $ZnSO_4 \cdot 7H_2O$ followed by centrifugation at 7500*g* for 10 min. Concentrations of the supernatants were measured with an HPLC system from Merck Hitachi (HPX 87-C-column from Aminex [85°C, 0.7 mL/min flow rate, bidistilled water as eluent], and an ERC 7512 IR-detector from Erma Cr. Inc.). External standard solutions of 10 g/L of lactose, galactose, and glucose were used for calculation.

Lactose conversion means the formed glucose measured by the Ebio glucose analyzer related to lactose determined by HPLC. The accuracy of glucose values obtained by the Ebio glucose analyzer was controlled by HPLC analysis of the same samples.

Results

Lactose Hydrolysis Studies With β-Galactosidase at 8 ± 2°C

Steady State

Using an experimental design in which skim milk (supplemented with 0.02% NaN_3) was pumped at a flow rate of 10.5 L/h and the enzyme solution (240 U/mL of Maxilact LX 5000) was circulated at 25 L/h, steady state was reached after 3 h. Lactose conversion in skim milk leveled at 50% and in the enzyme solution at 76%. Interestingly, after 60 min lactose conversion in skim milk was still higher than in the enzyme solution.

Enzyme Concentration-Dependent Lactose Conversion

Skim milk was supplemented with 0.02% NaN_3 and pumped at a rate of 10 ± 0.5 L/h. The lactose conversion rate at 120 U/mL was 44.12% and increased at 180 U/mL to 50.29%; at 240 U/mL to 56.77%; and, finally, at 300 U/mL to 63%. It is very important to recognize that the enzyme activity of 300 U/mL was still a limiting factor for the conversion rate. Adding more enzyme led to a further increase in lactose hydrolysis.

Skim Milk Flow Rate-Dependent Lactose Conversion

Figure 3 summarizes the results of the dependence of lactose conversion rate on the flow rate of experiments with an enzyme concentration of 240 U/mL at 8 ± 2°C. Continuous sterile filtration and 10 min/h UV irradiation at 45% intensity were performed. To point out the differences, the findings of the flow rate-dependent experiments at 23 ± 2°C (120 U/mL of enzyme activity) are included in Fig. 3. Lactose conversion at 8 ± 2°C in

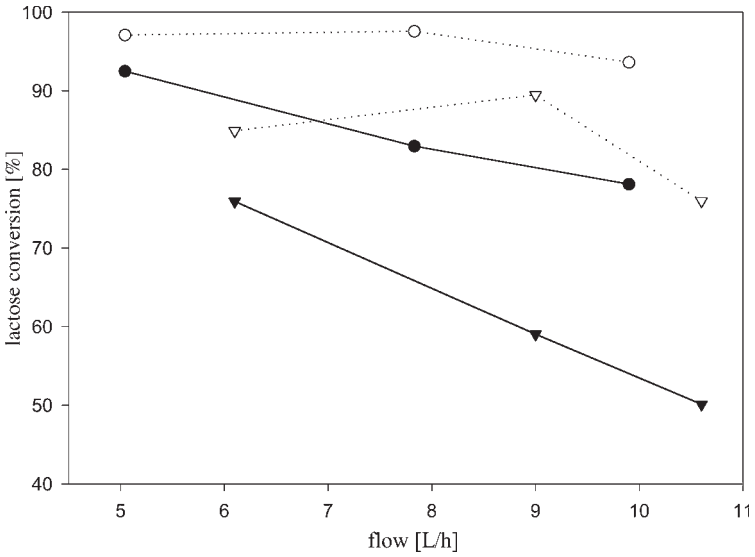

Fig. 3. Influence of skim milk flow rate on lactose conversion (%). Experiments at 8 ± 2°C were carried out with 240 U/mL of Maxilact L2000 and at 23 ± 2°C with 120 U/mL of Maxilact L2000 (flow rate of 25 L/h of enzyme solution). Lactose conversion in skim milk (●, 23 ± 2°C; ▼, 8 ± 2°C) and enzyme solution (○, 23 ± 2°C; ▽, 8 ± 2°C) are depicted. Skim milk was supplemented with 0.02% NaN_3.

skim milk decreased from 75.93% at 6.1 L/h to 59.04% at 9 L/h and further to 50.12% at 10.6 L/h; conversion rates determined in enzyme solution were always higher at the same flow rate. The resulting productivity was 222.32 g/(L·h) at 75.93% lactose conversion and skim milk flow rate of 6.1 L/h and was too low when considering the aim of 360 g/(L·h) of productivity.

Lactose Hydrolysis Studies With β-Galactosidase at 15 ± 2°C

Steady State

Steady state was reached after 150 min when skim milk (supplemented with 0.02% NaN_3) was pumped at a flow rate of 9.2 L/h and enzyme solution (240 U/mL of Maxilact L2000) circulated at 25 L/h. The measured lactose conversion leveled at approx 78% in skim milk and at 92% in enzyme solution.

Enzyme Concentration-Dependent Lactose Conversion

Skim milk was supplemented with 0.02% NaN_3 and pumped at 9 ± 0.5 L/h. Studies resulted in a pattern of enzyme concentration-dependent lactose conversion rates similar to that at 8 ± 2°C (Fig. 3), and an optimum enzyme concentration of 240 U/mL for lactose conversion of approx 75–80% was determined. Therefore, all further experiments at 15 ± 2°C were carried out with 240 U/mL of Maxilact.

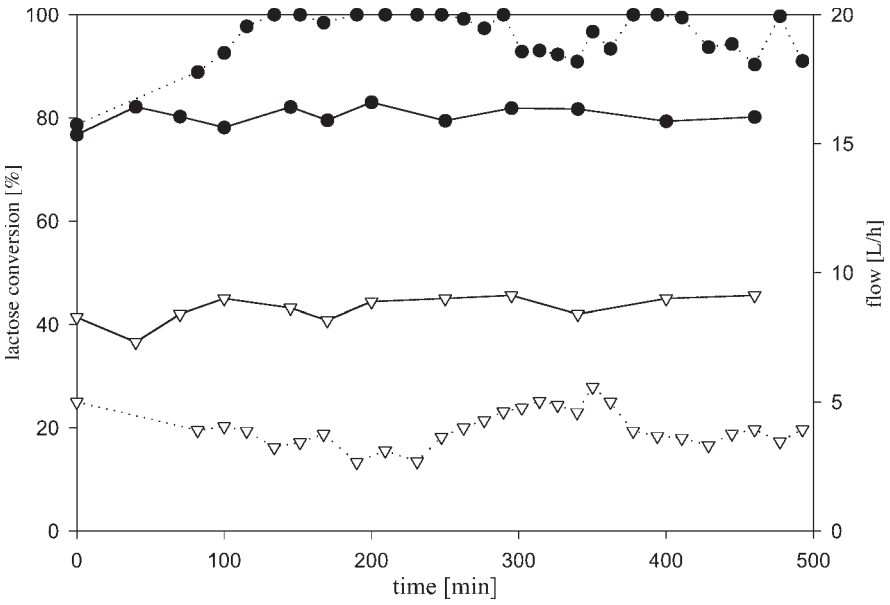

Fig. 4. Influence of skim milk flow rate on lactose conversion (%) at 15 ± 2°C. A comparison of two continuous production experiments aiming at 75% and at maximum lactose conversion is presented. Studies were carried out with 240 U/mL of Maxilact L2000 and at an enzyme solution flow rate of 25 L/h. Lactose conversion in skim milk (lined ● for 75% conversion rate, dotted ● for maximum conversion) and enzyme solution (lined ▽ for 75% conversion rate, dotted ▽ for maximum conversion) are depicted.

Long-Term Lactose Conversion Experiments

Figure 4 shows experiments at 15 ± 2°C in which neither sterile filtration nor UV irradiation was applied for 8 h. Maximum lactose conversion of 100% was achieved between skim milk flow rates of 3.6 and 3.8 L/h, whereas 81.86% was achieved at 9.12 L/h. The productivity aim of 360 g/(L·h) was nearly reached with 357.56 g/(L·h) at a flow rate of 9.12 L/h. Regarding process stability, no loss of enzyme activity was measured during these 8-h experiments, although sterile filtration and UV irradiation were not applied. After these findings, experiments were extended up to 20 h at 15 ± 2°C during which continuous sterile filtration and UV irradiation (10 min/h, 45% intensity) were applied only during the first 8 h and then were not used further. This experimental setting also resulted in no loss of enzyme activity.

Lactose Hydrolysis Studies With β-Galactosidase at 45 ± 2°C

Following the principle that enzyme activity and diffusion rates and, consequently, lactose conversion should be increased at higher temperatures, some temperature-dependent stability profiles for Maxilact L2000 were accomplished. These experiments showed quite good stability at 45°C. Thus, stability tests with a flow rate of 25 L/h of enzyme solution (120 U/mL

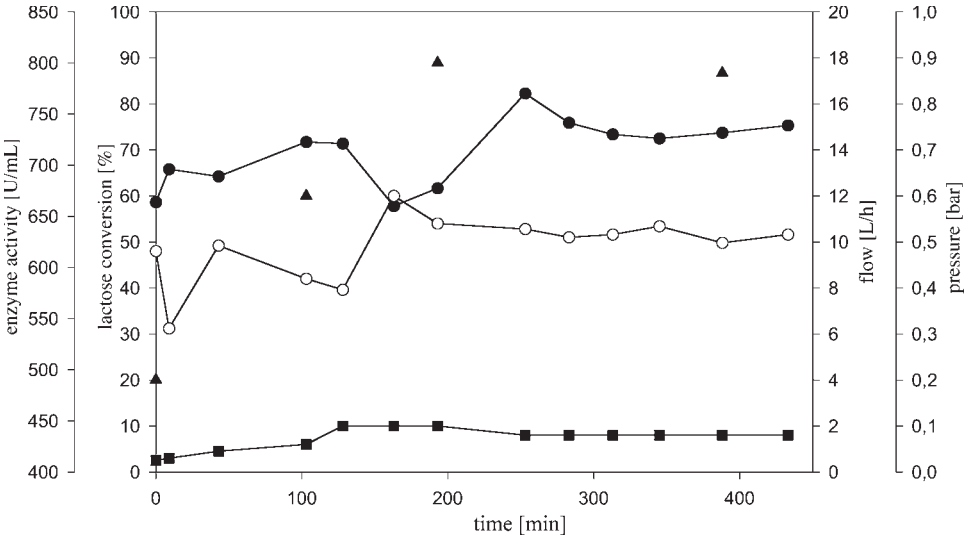

Fig. 5. Lactose conversion (%) experiment at 58 ± 2°C using thermostable β-glycosidase. The dependence of enzyme activity (▲) and skim milk flow rate (○) on lactose conversion rate (●) are depicted. Additionally, pressure (■) progression during this experiment was followed in order to detect blocking effects at the hollow-fiber module.

of Maxilact L2000 in skim milk permeate, 0.02% NaN_3) and a luminal flow of water of 10 L/h were carried out in the hollow-fiber plant. Luminal flow was applied in order to reach uniform heat distribution. After 4 h, enzyme activity dropped to zero, showing that these conditions were not suited for the production process.

Lactose Hydrolysis Studies With Recombinant β-Glycosidase (CelB) at 58 ± 2°C

Figure 5 represents a typical lactose hydrolysis experiment at 58 ± 2°C with β-glycosidase. Neither sterile filtration nor UV irradiation was performed. To demonstrate the dependence of lactose conversion on enzyme activity, enzyme concentration was increased up to 790 U/mL until the aimed productivity of 360 g/(L·h) was nearly achieved. During these experiments a small increase in pressure up to 0.11 bar was observed. Visual inspection after the production indicated that a higher proportion of milk protein had been luminally adsorbed in comparison to experiments at temperatures lower than 25°C. In addition, higher adsorption was detected in the heat exchangers, which were stresslessly cleaned with 0.1% NaOH.

Discussion

The aim of the present study was to develop and characterize a pilot plant process in order to obtain data for building an industrial plant. Novalin et al. *(12)* have previously shown that reaching the economic goal

Table 1
Summary of Temperature-Dependent Lactose Conversion Experiments[a]

Parameter	β-gal (8 ± 2°C)	β-gal (15 ± 2°C)	β-gal (23 ± 2°C)[b]	β-gal (45 ± 2°C)	β-gly (58 ± 2°C)
Enzyme concentration (U/mL)	240	240	120	ND	790
Flow rate (L/h)	6.10	9.12	9.90	ND	9.96
Steady state (min)	180	150	120	ND	ND
Lactose conversion (%)	75.93	81.86	78.11	ND	73.70
Productivity (g/L·h)	222.32	357.56	371.18	ND	352.34

[a]β-gal, β-galactosidase; β-gly, β-glycosidase; ND, not determined. Steady state accords to the time that was needed to establish constant production values under conditions described in the same column of this table.

[b]Data at 23 ± 2°C were reported by Novalin et al. *(12)*.

to develop a cheaper process than the batch process is possible by using hollow-fiber membranes with large membrane areas. In comparison to a batch process, the enzyme in the present membrane-based process has a longer stability and no enzyme is released in the product. Furthermore, the three-dimensional separation of the substrate from the enzyme solution minimizes blocking and washing-out effects, which restrict processes with immobilized enzymes. Another important economic property is long-term stability, in order to achieve cost-effectiveness for this process. In addition to the aimed productivity, the second goal was to prove a process stability of 20 h. To stabilize enzyme solution during this process, continuous sterile filtration and UV irradiation were performed. The same tests without sterile filtration or UV irradiation at 23 ± 2°C led to complete loss of enzyme activity and turbid enzyme solutions during an 8-h process time. Following the idea of finding a compromise between enhanced long-term stability of the process and higher costs, lactose conversion in the hollow-fiber bioreactor was characterized at 8 ± 2°C, 15 ± 2°C, 45 ± 2°C, and 58 ± 2°C. Table 1 summarizes the results of the temperature-dependent lactose conversion experiments.

Enzyme concentration-dependent experiments at 8 ± 2°C showed that the aimed 75% lactose conversion at 10 ± 0.5 L/h was not achieved with an enzyme concentration of 300 U/mL. Adding more enzyme led to a further increase in lactose hydrolysis, but it will result in higher cost. Czermak et al. *(8)* showed that this kind of process is mainly limited by lactose diffusion. Consequently, reaching a 75% conversion rate should be possible by flow rate reduction. This approach resulted in too low productivity of 222.32 g/(L·h) at a flow rate of 6.1 L/h. In comparison to 8 ± 2°C, diffusion rates and productivity would be increased at the same flow rate at 15 ± 2°C, when enzyme activity is not the limiting factor. Additionally, the growth rate of microorganisms should be significantly lower at 15 ± 2°C than at 23 ± 2°C. Investigations at 15 ± 2°C showed an enzyme concentration-dependent lactose conversion pattern similar to that at 8 ± 2°C resulting in an optimum

concentration of 240 U/mL for the aimed productivity of 360 g/(L·h). Additionally, 20 h of long-term process stability at 15 ± 2°C was achieved by applying UV irradiation and sterile filtration for only the first 8 h during the complete experimental course. This indicated that the proposed 20-h production running time could be extended and thus could lead to a higher cost-effectiveness.

Preliminary studies with β-galactosidase at 45°C promised appropriate enzyme stability in the bioreactor at the selected temperature. According to the process, limited growth of mesophilic microorganisms was expected at this temperature. However, studies with the hollow-fiber reactor showed that the enzyme activity was completely lost at a flow rate of the enzyme solution of 25 L/h after 4 h. It is probable that the additional shear stress caused by pumping reduced enzyme stability at this temperature.

Previous work by our research group *(14,15)* focused on developing a similar hollow-fiber process with thermostable β-glycosidase of *P. furiosus* (*CelB*). Establishing this kind of process obviously has further advantages. For example, lactose reduction of skim milk and pasteurization occur simultaneously. Loss of enzyme activity by microorganisms is significantly lower than at mesophilic conditions without sterile filtration or UV irradiation. A negligible growth of thermophilic germs is expected. Nevertheless, a remarkable difference of 10-fold higher stability of packed-bed *CelB* β-glycosidase in comparison to free enzyme solution was reported *(14)*. Pivarnik et al. *(3)* indicated that enzyme stability is one reason to favor packed-bed reactors instead of hollow-fiber reactors. The main cause for loss of enzyme activity in hollow-fiber reactors is the adsorption of the enzymes onto the membranes. To overcome this inherent problem, preadsorption processes with protein solutions in the shell side were carried out. Thus, loss of enzyme activity was reduced significantly. Experiments at 58 ± 2°C using β-glycosidase showed that the aimed productivity was almost achievable with 790 U/mL and at a skim milk flow rate of 9.96 L/h. However, hollow-fiber stability at elevated temperature has yet to be evaluated for process economy and to assess possible higher membrane blocking risks. In addition, the cost factor for producing β-glycosidase must be considered; bioreactor cultivation costs and the necessary high enzyme concentration (790 U/mL) may limit practicability according to the economic goals.

The data taken together suggest that first experiments with an industrial plant should be carried out at 15 ± 2°C because of the expected longer production running times and smaller microorganism's strain. Decreasing the temperature to 8 ± 2°C would not lead to the desired productivity without applying high enzyme concentrations; tests at 58 ± 2°C showed higher blocking risks and nonpredictive enzyme costs. Compared to 23 ± 2°C, a doubled enzyme concentration must be used at 15 ± 2°C, but this can be compensated through longer stability and utilization of the enzyme's activity.

Conclusion

The major conclusions of this study are as follows: First, the optimization studies for the innovative hollow-fiber process to reduce lactose in skim milk showed that the aimed productivity of 360 g/(L·h) was achievable at temperatures of 15 ± 2°C (240 U/mL of Maxilact), 23 ± 2°C (120 U/mL of Maxilact), and 58 ± 2°C (790 U/mL of β-glycosidase) in the pilot plant. Second, the main problem lies in the growth of microorganism within the enzyme solution. Steam-sterilizable hollow-fiber reactors would be too expensive for a cost-effective process. The installation of a sterile filtration module and application of UV irradiation reduced microbial stress at 23 ± 2°C. A further decrease in temperature to 15 ± 2°C improved long-term stability.

In any case, the applicability of membrane-diffusion reactors needs to be tested commercially. It was possible to introduce and optimize a process for direct lactose hydrolysis in skim milk without any ultrafiltration step before enzymatic conversion. Furthermore, this system can be easily connected with milk storage tanks in the dairy industry as an inline installation. Additionally, it should be possible to use other substrates for this process, such as whey or high-concentrated whey. In comparison to a batch process, parameters were achieved that could be the basis for an economic process.

Acknowledgments

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