Design and Economics of Industrial Production of Fructosyltransferase*

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Received 1 April 2005

A production process of preparation FTase, a powdery purified enzyme, from *Aureobasidium pullulans* was designed. The process flowsheet consisted of unit operations needed for the aerobic cultivation of the cells and subsequent separation steps of the enzyme recovery from the cultivation medium and cell mass. An optimum schedule of operations was designed for the defined annual production capacity that minimized the number of equipment pieces and maximized the time of their usage. The evaluated mass and energy balances and calculated dimensions of the equipment formed the basis for the calculation of capital and operating cost of the production process. The profitability of the designed process was analyzed as a function of the price of the enzyme preparation.

Fructosyltransferase (FTase) is an enzyme that catalyzes the transformation of sucrose into fructooligosaccharides, which are important prebiotic compounds having a broad application in food and pharmaceutical industries. Fructosyltransferase catalyzes the transfer of fructosyl moieties where a donor or acceptor of these moieties can be sucrose or fructooligosaccharides [1]. In the industrial production of fructooligosaccharides, the cells with the FTase activity are produced by aerobic cultivation of fungi such as *Aspergillus niger* [2], *Aspergillus japonicus* [3], or *Aureobasidium pullulans* [4]. They are applied for the biocatalytic process in immobilized form.

In our laboratory, we have dealt with the development and optimization of the process of cultivation of the cells of *A. pullulans* with the FTase activity [5—8]. The increasing interest in prebiotic compounds opens also possibilities for small-scale use of FTase. Isolated enzyme could be a suitable form for such purposes. For that reason, we have also recently dealt with the downstream processing of FTase from the broth obtained at the cultivation of *A. pullulans* [9]. The obtained data can be used for the design of the production process of FTase and analysis of its economic efficiency.

The design and scheduling of industrial biotechnological process is often simplified by specialized computer-aided software such as Aspen Batch Plus or SuperPro-Designer [10]. These were applied in several studies of scale-up, optimal plant design, and analysis of investment and operating costs of pilot and industrial production of proteins. The examples include the production of insulin [11, 12], tissue plasminogen activator [13, 14], β-galactosidase [15], heparinase [16], or growth hormone [17].

The objective of this publication was to design the industrial production of FTase from cultivation to final treatment based on experimental and literature data and to evaluate the economic balance of the production process. At first, a sequence of suitable operations for isolation and purification of FTase had to be suggested, which would consider the character of the source material, production form of enzyme, or level of product purity. All suggested operations had to provide suitable conditions for retaining of the biological activity of FTase. Then, it was necessary to define suitable equipment for particular operations and to calculate material and energy balances for all processes. For an effective utilization of the equipment, the time schedule of operations was created. The investment and operating costs could finally be calculated for the assessment of the effectiveness of the process design.

PROCESS DESIGN

FTase of *A. pullulans* occurs in the periplasmic space of cells and so the part of the enzyme is easily released to the cultivation medium. Therefore, the recovery of the enzyme was considered from both the

*Presented at the 32nd International Conference of the Slovak Society of Chemical Engineering, Tatranské Matliare, 23—27 May 2005.
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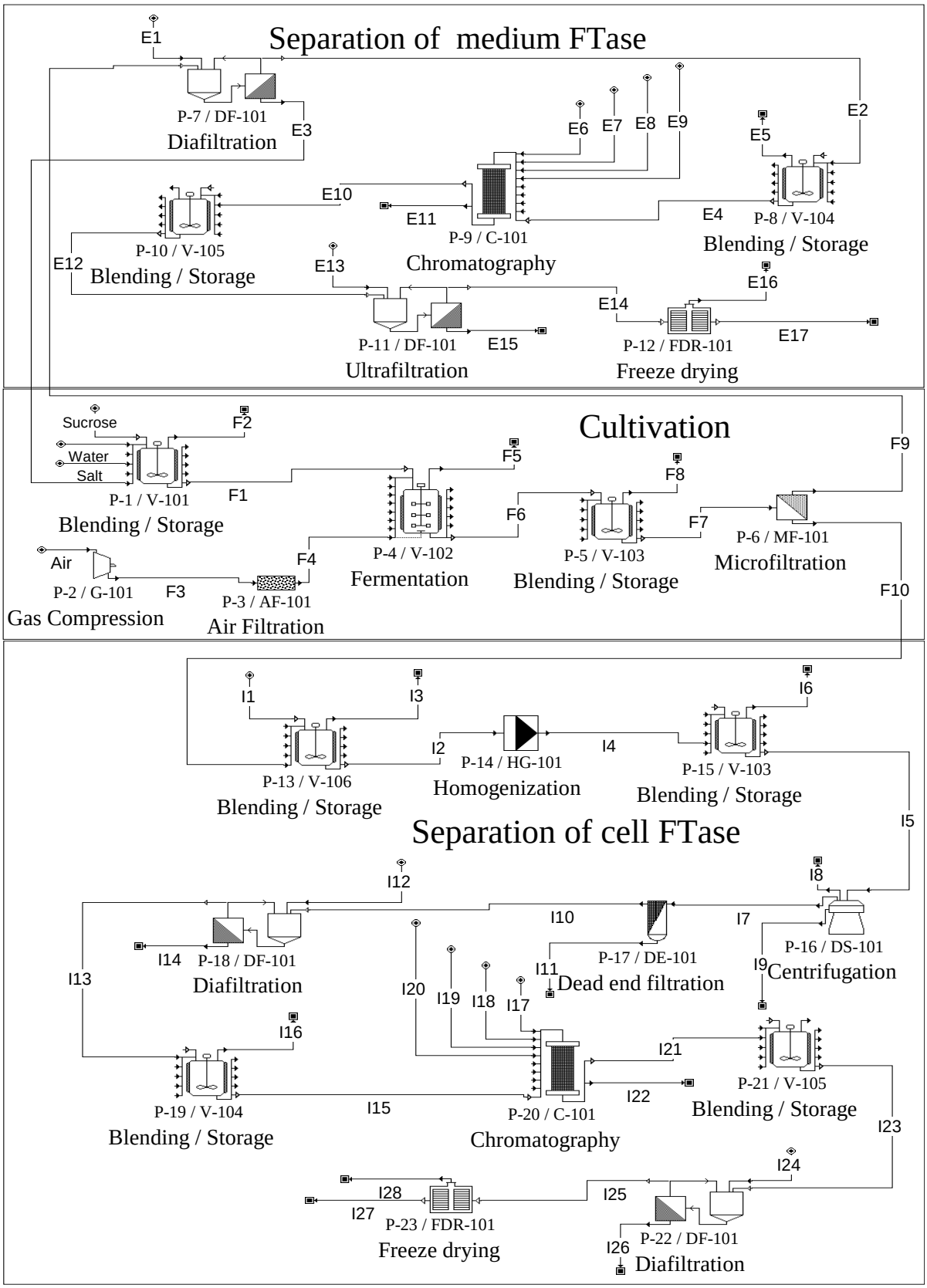

Fig. 1. Process flowsheet of industrial production of FTase. Labels of the equipment units are in the form Number of procedure/Type of equipment in the SuperPro-Designer. Streams are denoted by alphanumeric codes where F denotes the cultivation part, E the part of the cell FTase separation, and I the part of the medium FTase separation.

Table 1. Characteristics of Equipment and Operations Used and Compositions of Outlet Streams

Equipment	Code	Operation	Duration time/h	Temperature/ °C	Stream	Composition/mass %
Blending Tank	V-101	P1	1.5	25	F1	Salts 2.51, Sucrose 24.63, $NaNO_3$ 3.45, Water 69.37
Bioreactor	V-102	P4	80	28	F6	Biomass 7.32, FTase 0.0024, Proteins 0.46, Salts 2.69, Sucrose 12.76, Water 76.78
Microfilter	MF-101	P6	2.5	12	F9	FTase 0.0038, Proteins 0.72, Salts 4.24, Sucrose 20.12, Water 74.92
					F10	Biomass 19.83, Proteins 0.01, Salts 0.04, Sucrose 0.17, Water 79.96
Blending Tank	V-106	P13	2.4	11	I2	Biomass 7.47, Salts 0.01, NaCl 0.53, Sucrose 0.06, Water 91.91
High-Pressure Homogenizer	HG-101	P14	7.9	12	I4	Biomass 0.04, Debris 1.48, FTase 0.0035, Proteins 0.08, Salts 0.01, NaCl 0.53, Sucrose 0.06, Water 97.78
Disk-Stack Centrifuge	DS-101	P16	7.7	13	I7	Debris 0.60, FTase 0.0070, Proteins 0.11, Salts 0.02, NaCl 0.77, Sucrose 0.09, Water 98.40
Dead-End Filter	DE-101	P17	1.7	13	I10	FTase 0.0072, Proteins 0.11, Salts 0.02, NaCl 0.78, Sucrose 0.09, Water 98.90
Diafilter	DF-101	P7	2.0	12	E2	Citric acid 0.01, FTase 0.0406, Na_2HPO_4 0.01, Proteins 1.72, Salts 0.62, Sucrose 2.94, Water 94.66
		P11	0.7	10	E14	Citric acid 0.01, FTase 0.0676, Na_2HPO_4 0.01, Proteins 0.28, NaCl 0.04, Water 99.60
		P18	1.6	12	I13	Citric acid 0.01, FTase 0.0650, Na_2HPO_4 0.01, NaCl 0.10, Proteins 0.23, Sucrose 0.01, Water 99.58
		P22	0.47	12	I25	Citric acid 0.01, FTase 0.1370, Na_2HPO_4 0.01, Proteins 0.05, NaCl 0.04, Water 99.76
Chromatographic Column	C-101	P9	15.9	10	E10	Citric acid 0.14, FTase 0.0134, Na_2HPO_4 0.19, Proteins 0.09, NaCl 0.88, Water 98.70
		P20	11.2	10	I21	Citric acid 0.14, FTase 0.0272, Na_2HPO_4 0.19, NaCl 0.88, Proteins 0.01, Water 98.75
Freeze-Dryer	FDR-101	P12	10	−4	E17	Citric acid 1.72, FTase 16.7225, Na_2HPO_4 2.33, Proteins 68.36, NaCl 10.87
		P23	10	−4	I27	Citric acid 2.86, FTase 56.3560, Na_2HPO_4 3.88, Proteins 18.82, NaCl 18.09

harvested cells and cultivation medium. The goal was to design an industrial procedure of isolation and purification providing a powdery product with a specific enzyme activity.

The production flowsheet shown in Fig. 1 consists of three parts: cultivation, separation of FTase from cells, and separation of FTase from a medium. Table 1 summarizes the individual operations of the flowsheet together with their basic characteristics and compositions of outlet streams.

The cultivation medium was prepared in a tank (P1/V-101) and transferred into one of the two installed bioreactors (P4/V-102). The compressor G-101 and the air filter AF-101 ensured sterile air for the cul-

Table 2. Overview of Operation Steps of Chromatographic Purification of FTase

Process	Input stream	Stream description	t/h	Volume/dm^3
Loading	E8	Retentate of diafiltration	0.11	36
Column wash	E9	Buffer A*	0.38	119.58
Column elution	E9 + E10	Buffer A + buffer B**	0.18	143
Column wash	E10	Buffer B***	0.24	95
Regeneration 1	E11	NaOH (0.5 mol dm^{-3})	0.12	47
Regeneration 2	E12	Water	0.27	190
Equilibration	E9	Buffer A	0.33	240

* Buffer A: 0.02 mol dm^{-3} phosphate-citrate buffer, pH 6.0.
** Buffer A and buffer B in the volumetric ratio 85:15.
*** Buffer B: 1 mol dm^{-3} NaCl in buffer A.

Table 3. Yield and Purification Factor of Isolation and Purification of Medium FTase

Operation	Equipment	Total activity × 10^{-6}	Specific activity*	FTase yield	Purification factor
		U	U g^{-1}	%	
Microfiltration	P6/MF-101	746	17 215	100	–
Diafiltration	P7/DF-101	746	76 039	100	4.4
Chromatography	P9/C-101	634	440 817	85	25.6
Ultrafiltration	P11/DF-101	634	648 210	85	37.7
Lyophilization	P12/FDR-101	634	648 210	85	37.7

*Specific activity is the FTase activity per total mass of proteins.

Table 4. Yield and Purification Factor for Isolation and Purification of Cell FTase

Operation	Equipment	Total activity × 10^{-6}	Specific activity*	FTase yield	Purification factor
		U	U g^{-1}	%	
Microfiltration	P6/MF-101	4*	18 748	–	–
Storage	P13/V-104	4*	18 748	–	–
Homogenization	P14/HG-101	1083	145 198	100	–
Centrifugation	P16/DS-101	1061	199 116	98	1.4
Dead-end filtration	P17/DS-101	1058	199 116	98	1.4
Diafiltration	P18/DF-101	1058	739 297	98	6.3
Chromatography	P20/C-101	900	2 158 593	83	35.8
Ultrafiltration	P22/DF-101	900	2 475 070	83	65.1
Lyophilization	P23/FDR-101	900	2 475 070	83	65.1

* Extracellular FTase is included in the liquid phase.

tivation at an average rate of 1 m^3 of air per minute per 1 m^3 of medium. At the end of cultivation, the content of bioreactors was transferred into a tank (P5/V-103) and thereafter into a microfilter (P6/MF-101). In the microfilter, two product streams were formed and the flowsheet was split here into two paths when FTase was contained in both the filtrate and cell retentate.

The filtrate, F9, was further processed in a diafilter (P7/DF-101), where low-molecular-mass solutes (salts, sucrose, and a part of proteins) were removed, water was exchanged with buffer A and the FTase concentration increased by a factor of 3. The stream E3 containing low-molecular-mass substances from the diafilter was re-used in the cultivation. FTase in the solution was further purified in two ion-exchange chromatographic columns (P9/C-101) with the total binding capacity of chromatography resin of 20 mg cm^{-3}. Table 2 presents an overview of individual steps of the chromatographic process where the most of the unwanted proteins were removed but the FTase in the product stream, eluate E10, was significantly diluted by water. The large volume of water was consequently reduced by ultrafiltration (P11/DF-101), which took place in the same equipment as diafiltration. The final powdery product, E17, with the FTase activity of 648 000 U g^{-1} of protein (Table 3) was obtained in a freeze dryer (P12/FDR-101). The yield of FTase recovery from the medium was 85 % and the purification factor with respect to the initial total protein content was 37.7. Table 3 also illustrates the separation efficiencies of individual operations.

The cell concentrate, F10, with the composition

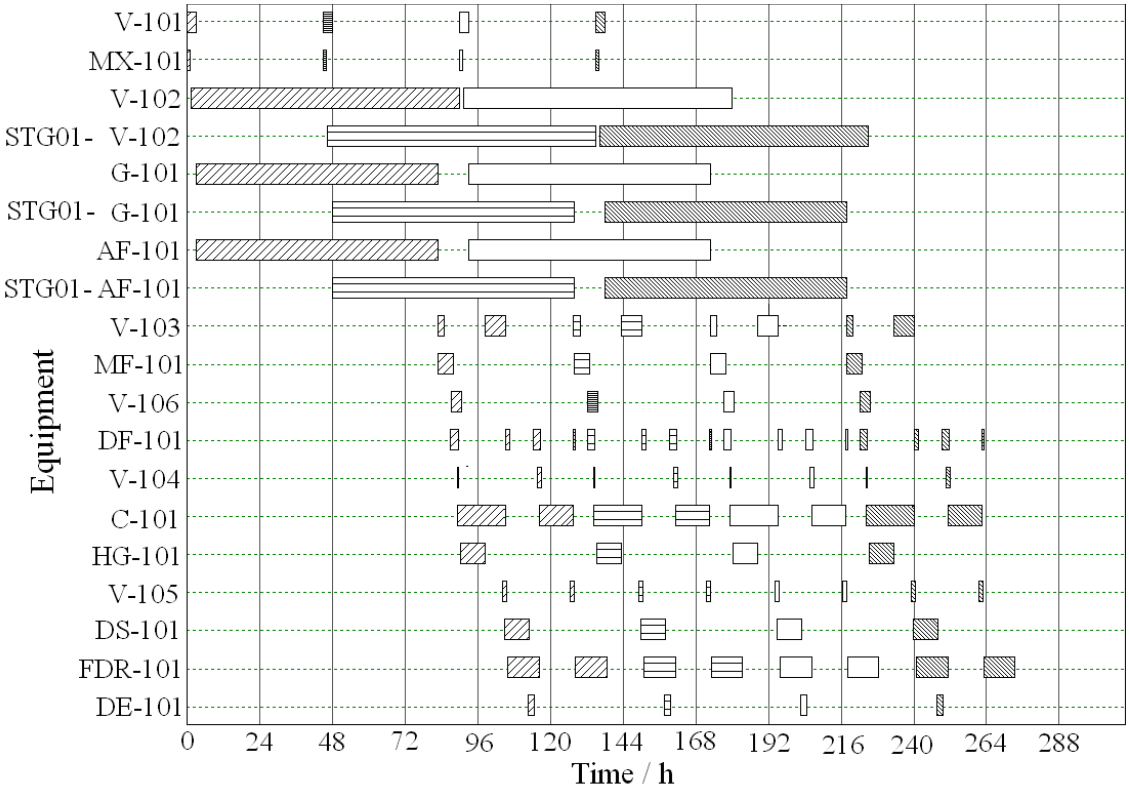

Fig. 2. Diagram of the utilization of the equipment in four consecutive batches. The first cycle in the first bioreactor ▨, the first cycle in the second bioreactor ▤, the second cycle in the first bioreactor ▯, and the second cycle in the second bioreactor ▧.

given in Table 1 was an input stream for the separation of cell FTase. After dilution with a buffer, the cell suspension passed into a high-pressure homogenizer (P14/HG-101). The cells were here disrupted and the intracellular content was released into the liquid phase. The efficiency of homogenization with respect to FTase was 95 %. Solids (undisrupted cells and cell debris) were then removed by centrifugation (P16/DS-101) and dead-end filtration (P17/DE-101). Almost all the cells and 80 % of debris were removed by centrifugation. The remaining cell debris was removed by dead-end filtration. Clarified liquid was further treated in the equipment used also for the FTase separation from the medium.

Details of individual operations are given in Tables 1 and 2. The powdery enzyme I27 obtained in this part of the plant had the specific FTase activity of 2 475 000 U g^{-1} (Table 4). The yield and the purification factor of FTase were 83 % and 65.1, respectively. The final product obtained by the mixing of the enzyme streams E17 and I27, had a specific FTase activity of 1 143 000 U g^{-1} and contained 28.4 % of FTase.

Fig. 2 shows that the duration of one batch cycle for this process design was 139 h. The time schedule of individual operations presented in this figure provided an average degree of utilization of the equipment of 42 %. The bottleneck operation was the cultivation lasting 90 h, which would determine the time between the starts of two consecutive batch cycles. Applying the throughput analysis using the software SuperPro-Designer (Intelligen Inc., Scotch Plains, NJ, USA), a plant with two bioreactors was designed, which decreased the mentioned time to 45 h (Fig. 2). The equipment usage then increased to 85 %. The plant was designed for the annual operation time of 7920 h and the production capacity of 80 kg of FTase (100 %) that resulted in 173 batches.

ECONOMICS

SuperPro-Designer was also used for the economic analysis of the process design. The costs of equipment were obtained from the local representatives of Ayton Equipment Limited Station Yard (Middlesbrough – Cleveland, England). Table 5 shows the list of equipment used in the process design with basic specifications and costs. The most expensive piece of equipment was the freeze-dryer, which formed about 30 % of the total equipment cost (Table 6). Two bioreactors and chromatographic columns formed another third of the total equipment cost. Table 6 further shows that the total equipment cost was 2.6 million EUR when the cost of the equipment unlisted in Table 5 was 465 000 EUR. The equipment cost was the basis for calculation of other costs composing the fixed capital investment such as installation, instrumentation, *etc.* These costs, shown in Table 6, were calculated by multiplication of the equipment cost by appropriate

Table 5. List of the Equipment Used

Name	Description	Cost/EUR
V-104	Blending Tank, Vessel Volume = 1.2 m^3	27 000
DF-101	Diafilter, Membrane Area = 50.0 m^2	95 000
C-101	Chromatographic Column, Volume = 47.7 dm^3, 2 pieces	215 000
FDR-101	Freeze-Dryer, Sublimation Capacity = 283 kg h^{-1}	803 000
V-106	Blending Tank, Vessel Volume = 10.0 m^3	52 000
HG-101	Homogenizer, Rated Throughput = 3.50 m^3 h^{-1}	38 000
DS-101	Disk-Stack Centrifuge, Throughput = 100.00 dm^3 min^{-1}	88 000
DE-101	Dead-End Filter, Filter Area = 15.00 m^2	32 000
MF-101	Microfilter, Membrane Area = 30.00 m^2	44 000
V-102	Bioreactors, Vessel Volume = 10.0 m^3, 2 pieces	506 000
AF-101	Air Filter, Rated Throughput = 0.06 m^3 s^{-1}, 2 pieces	13 000
G-101	Centrifugal Compressor, Power = 400 kW, 2 pieces	63 000
V-101	Blending Tank, Vessel Volume = 10.0 m^3	52 000
V-105	Blending Tank, Vessel Volume = 6.0 m^3	42 000
V-103	Blending Tank, Vessel Volume = 10.0 m^3	52 000

Table 6. Summary of the Fixed Capital Investment

Total Plant Direct Cost (TPDC)		Price/EUR
1	Equipment Purchase Cost	2 585 000
2	Installation	582 000
3	Process Piping	775 000
4	Instrumentation	569 000
5	Insulation	77 000
6	Electricity	258 000
7	Buildings	517 000
8	Yard Improvement	388 000
9	Auxiliary Facilities	775 000
TPDC		6 528 000
Total Plant Indirect Cost (TPIC)		
10	Engineering	979 000
11	Construction	1 631 000
TPIC		2 600 000
Total Plant Cost (TPC = TPDC+TPIC)		
TPC		9 138 000
Contractor's Fee & Contingency (CFC)		
12	Contractor's Fee	183 000
13	Contingency	731 000
CFC = 12+13		914 000
Direct Fixed Capital Cost (DFCC = TPC+CFC)		
DFCC		10 052 000

Table 7. Operating Cost of FTase Production

Cost Item	Cost/EUR
Raw Materials	5 410 000
Facility-Dependent	1 301 000
Consumables	769 000
Utilities	364 000
Waste Treatment/Disposal	328 000
Labour-Dependent	231 000
Laboratory/QC/QA*	162 000

*QC/QA – quality control and quality assurance.

factors [18]. The fixed capital investment was then 10 million EUR. The total capital investment contained besides the fixed capital investment the working capital and start-up costs. The working capital, the investment in temporary or consumable materials, was 892 000 EUR, which was 8.9 % of the fixed capital investment. The start-up cost associated with the start-up and validation of the process represented 5 % of the total capital investment. The total capital investment was thus 11.4 million EUR.

The costs of raw materials were provided by the local vendors of chemicals, SLAVUS s.r.o. (Bratislava, Slovakia) and CHEMIKA-NEUBER Slovakia s.r.o. (Pezinok, Slovakia). The calculated annual cost of raw materials was 5.1 million EUR. It formed 63 % of the annual operating costs amounting to 8.2 million EUR (Table 7). Table 7 also lists other operating costs. The second largest item was the facility-dependent cost that included local taxes and maintenance, insurance, and depreciation of equipment. The depreciation was calculated over a 10-year period assuming a 5 % salvage value for the entire production. The consumables included the costs of replacement filter cloths and membranes, chromatography resins, *etc.* They formed 9 % of the annual operating costs. The utilities comprised heating, cooling, and electricity consumption. The waste treatment included liquid waste and emissions. Emissions contained nitrogen, oxygen, and carbon dioxide and they were not dangerous for the environment. The liquid waste consisted of proteins, sucrose, and salts dissolved in water that could be treated by biological wastewater treatment. The labour-dependent cost included personnel costs except of those that were a part of the costs for

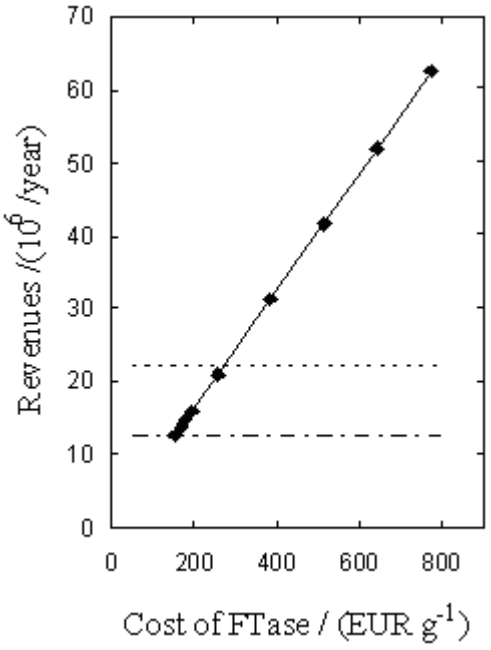

Fig. 3. Variation of revenues *vs.* the price of FTase. The dotted line indicates the revenues needed for the payback time of 2 years and the dotted-dashed line the payback time of 4 years.

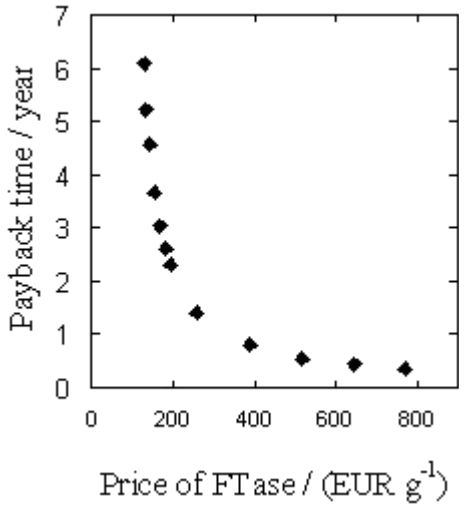

Fig. 4. Payback time *vs.* the price of FTase.

laboratory, quality control, and quality assurance.

Since fructosyltransferase has not been marketed yet, an analysis of the investment returns with respect to the chosen price was performed. Fig. 3 presents the calculated dependence of annual revenues on the price of FTase, which was in the range of the prices of other common hydrolytic enzymes of comparable purity and activity for laboratory use. Two horizontal lines in the figure delineate the payback periods of two and four years, respectively. The payback period is plotted as a function of the FTase price in Fig. 4. An exponentially decreasing function was obtained which indicates a critical FTase price of about 200 EUR g^{-1}. At this price, the payback time was approximately 2 years. Even if one assumes that the accuracy of the estimated design is about 20—30 %, this price could guarantee a safe profitability of the production. Below the price of 200 EUR, the payback time increases significantly and the investment return could be threatened. On the other hand, the calculated production costs were conservative. They could be reduced if partly or fully depreciated equipment is available. Furthermore, the use of multipurpose plant could be considered for this production where the risk of overestimation of the expected demand for the product could be significantly reduced. It could be further considered that the plant could provide several FTase products with different, lower degrees of purity in the liquid form. Such cheaper products could be considered both for laboratory and small-scale industrial use.

Acknowledgements. This study was supported by the Slovak Grant Agency for Science VEGA (Grant No. 1/0065/03). Mr. Martin Minárik from LIKO Bratislava a.s. is kindly acknowledged for useful advices.

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