



ATB
Agrartechnik Bornim



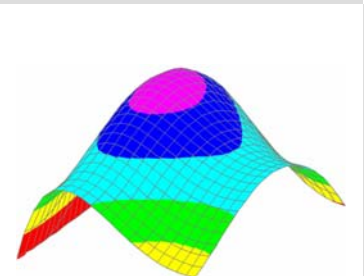
Biochemicals and Energy from Sustainable Utilization of herbaceous Biomass (BESUB)



Biochemikalien und Energie
aus der nachhaltigen Verwertung
pflanzlicher Biomasse



Sustainable development, global change and ecosystems
Sustainable energy systems



Bornimer Agrartechnische Berichte

Heft 42

Potsdam-Bornim 2005



Biochemicals and Energy from Sustainable Utilization of herbaceous Biomass (BESUB)

Biochemikalien und Energie aus der
nachhaltigen Verwertung pflanzlicher
Biomasse



Sustainable development, global change and ecosystems
Sustainable energy systems

Herausgeber:

Leibniz-Institut für Agrartechnik Potsdam-Bornim e.V. (ATB)
Max-Eyth-Allee 100
14469 Potsdam

☎ 0331 5699-0
Fax 0331 5699-849
E-Mail atb@atb-potsdam.de
Internet www.atb-potsdam.de

Bearbeiter des ATB:

Dr.-Ing. Joachim Venus
Dr. sc. nat. Dr. rer. nat. Klaus Richter
Dr.-Ing. Dr. sc. agr. Winfried Reimann
Dipl.-Ing. (FH) Christine Mehlmann
Dipl.-Ing. (FH) Rohland Schneider

Projekt:**General Project Information**

www.cordis.lu/eesd/src/projects.htm

CRAFT Contract: ENK-CT-2002-30014
CRAFT Project No: CRAF-1999-70986

Projektpartner:**SME:**

- IBC - Icelandic Biomass Company, Hafnafjordur (ISL)
- Beltra Forestry Ltd., New Port (IRL)
- tetra Ingenieure GmbH, Neuruppin

RTD:

- RALA Agric. Res. Inst., Reykjavik (ISL)
- Universität Heidelberg
- Biorefinery.de, Potsdam
- BIOPOS, Teltow
- Institut für Agrartechnik Bornim (ATB)

Typographische Gestaltung:

Joachim Venus
Regina Hager

Oktober 2005

Herausgegeben vom Leibniz-Institut für Agrartechnik Potsdam-Bornim e.V. (ATB) mit Unterstützung der Europäischen Kommission.

Für den Inhalt der Beiträge sind die Autoren verantwortlich.

Eine Weiterveröffentlichung von Teilen ist unter Quellenangabe und mit Zustimmung des Leibniz-Instituts für Agrartechnik Potsdam-Bornim e.V. möglich.

ISSN 0947-7314

© Leibniz-Institut für Agrartechnik Potsdam-Bornim e.V., Potsdam-Bornim 2005

Content

Project summary.....	5
1 Introduction.....	8
2 Strain selection and strain characterization	10
2.1 Objectives	10
2.2 Screening program	10
2.3 Preliminary assessment of fermentation performance	12
2.4 Phenotypic strain optimisation.....	13
2.4.1 Methods	13
2.4.1.1 Experimental design	14
2.4.1.2 Conditions of cultivation.....	15
2.4.1.3 Test description	15
2.4.1.4 Evaluation of experiments	15
2.4.2 Results	20
2.4.2.1 Characteristic quantities related directly from the measured data	20
2.4.2.2 Estimate of kinetic values for product formation and cell growth from experimental data by model approximation	21
2.4.2.3 Estimation of the optimal parameters for lactic acid production and cell growth by means of non-linear regression	23
2.4.2.4 Estimate of the maximum values of the specific performance rates of the strain <i>L. paracasei</i> 168 by means of non-linear regression.....	27
2.4.3 Discussion of results and conclusions	30
3 Preparation of glucose from barley grains	36
3.1 Grain milling	36
3.2 Enzymatic hydrolysis of barley coarse meal	37
3.2.1 Starch liquefying	37
3.2.2 Starch saccharification.....	41
3.3 Mass balance of starch hydrolysis	46
4 Utilization of green biomass as nitrogen source	47
4.1 Preparation of green juice from wet lucerne by pressing	47
4.2 Chemical analysis of pressing products.....	48

5	Batch fermentation in laboratory and bench scale	51
5.1	Raw materials for lactic acid fermentation	51
5.2	Batch cultivation of a lactic acid bacterium on barley hydrolysate	52
5.3	Batch cultivation of a lactic acid bacterium on lucerne green juice	54
5.4	Lactic acid production in bench scale	57
6	Continuous lactic acid fermentation process	58
6.1	Operation in chemostat mode	58
6.2	Cell recycle bioreactor	59
6.3	Conclusions	61
7	Down-stream processing of lactic acid	62
7.1	Methods	62
7.1.1	Membrane filtration	62
7.1.2	Removal of divalent cations	63
7.1.3	Electrodialysis	64
7.1.4	Mono-polar electrodialysis	65
7.1.5	Bipolar electrodialysis	65
7.1.6	Ion exchange	66
7.1.7	Analytical Methods	66
7.2	Results and Discussion	67
7.2.1	Ultrafiltration	67
7.2.2	Removal of multivalent metals	68
7.2.3	Conventional electrodialysis (EDM)	69
7.2.4	Bipolar electrodialysis (EDB)	70
7.2.4.1	Two-compartment bipolar electrodialysis (EDB2C)	71
7.2.4.2	Three-compartment bipolar electrodialysis (EDB3C)	72
7.2.5	Final purification stages	72
7.3	Conclusions	74
8	References	75
9	List of Figures	78
10	List of Tables	81

Project summary

The explosive growth of industrial societies in the last century was achieved through scientific and technological development, and easy access to cheap energy like fossil fuels. Social and environmental imbalances resulted and employment opportunities concentrated to urban centres bringing about high levels of pollution concentration, fertiliser and pesticide contamination and global warming. Sustainable development, which in political and public terms primarily means careful resource management and greater focus on renewable energy developments, has become the catchphrase at the start of the 21st century. To meet this challenge the project partners, which are involved in designing bio-refineries using biomass such as grains, grasses, legumes and straw for feedstock, have decided to engage in the production of the following industrial bio-products:

- Transportation fuels from biomass: e.g. ethanol.
- High value bio-chemicals and materials from biomass-based raw materials e.g. sparteine derivatives, ethyl-lactate.
- Animal feed based on starchy and cellulosic feedstock.
- Carbon dioxide for the preservation of foods.
- Biogas production for use in electric utilities, industry and for private use.

Project objectives

- Ascertain the availability of suitable biomass, it's cost, quality and yields
- Develop methodology and processes for extracting high value bio-chemicals from green biomass such as sparteine and it's derivatives
- Develop a new continuous fermentation process for lactic acid and a new ethyl-lactate production process
- Select optimal economical processes and products that contribute to the biosphere and assess production in rural communities

Project structure

The project partnership is composed of partners from three countries

<u>SME:</u>	<u>RTD:</u>
- IBC - Icelandic Biomass Company, Hafnafjordur (ISL)	- RALA Agric. Res. Inst., Reykjavik (ISL)
- Beltra Forestry Ltd., New Port (IRL)	- Universität Heidelberg
- tetra Ingenieure GmbH, Neuruppin	- Biorefinery.de, Potsdam
	- BIOPOS, Teltow
	- Institut für Agrartechnik Bornim (ATB)

and of different of activities:

WORK PACKAGES	SHORT DESCRIPTION
WORK PACKAGE WP1:	Biomass procurement
WORK PACKAGE WP2:	Separation of sparteine and development of derivatives
WORK PACKAGE WP3:	Fermentation and Esterification
WORK PACKAGE WP4:	Further use of fibre and liquid residue after extraction, fermentation and protein recovery
WORK PACKAGE WP5:	Economic assessment
WORK PACKAGE WP6:	Project evaluation and proposal for further steps
WORK PACKAGE WP7:	Project Co-ordination and dissemination

Bio-refinery

A bio-refinery is a processing plant in which biomass feedstock is converted into a spectrum of valuable products with zero or near zero CO₂ emission in its overall concept. Bio-refineries are based upon a similar concept as petrochemical refinery technologies, but are much more complex and offer significantly greater potential because the quantity of renewable biomass is enormous. Moreover, they offer the possibility of being non-polluting industries in terms of biodegradability and zero emission of harmful wastes and greenhouse gases. Their development will result in keys for the integrated production of chemicals, animal feed and fuels as outlined in **Figure 1**.

The bio-refineries proposed will provide high-technology jobs in rural environment, as well income to farmers from the production of biomass. In general energy gains will exceed energy use. The processes developed will be implemented in the near future by the participating SME's involved in this project. It will be licensed to qualified parties and the products sold mainly through professional vendors.

The BESUB Craft-project considered and projected a number of bio-refinery options using herbaceous biomass. Two main initial implementation approaches remain feasible, one based on the use of rye grain (ATB-tetra) for the production of ethyl-lactate, the other based on the use of barley/lupine to produce ethanol and by-products, such as ethyl lactate and animal feed.

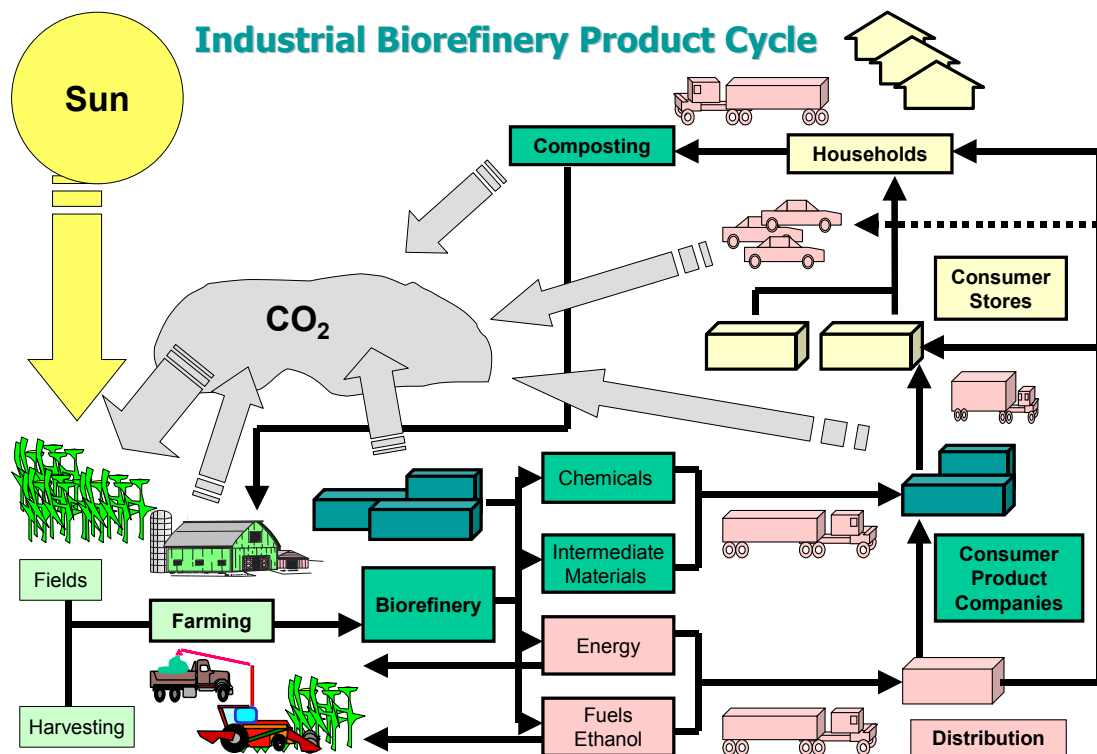


Figure 1: Sustainable Biorefinery Products Cycle (without foods)

Project approach

Being a pan-European project, a specific goal of this project has been to focus on widely available herbaceous feedstock. The Alaska lupine has been used for land reclamation purposes with very good results. Large expanses of barren land, which had reached quite serious levels of land erosion, have been reclaimed.

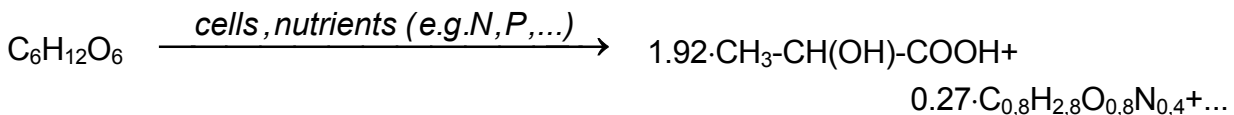
The most relevant data needed for commercial harvesting of these plants for biomass purposes such as sustainability of growing, feasibility of harvesting, suitability etc. has been investigated. Special focus was directed towards the lupine, which contains the alkaloid sparteine, which has potential uses as a natural insecticide. The feasibility of using the types of biomass available has been assessed based on present knowledge with emphasis on cost in term of kg DM (Dry Matter). The growing of *Lupinus Nootkatenisis* has a good potential on the extensive sandy areas on the southern coast of Iceland.

The aim of ATB's research is to create a new lactic acid production process using starchy crops (grains, potatoes) and green plant materials (lucerne, lupine) as raw materials. In collaboration with the Potsdam Research Group (Biopos), the process will be adapted to an esterification process for producing ethyl lactate. Use of by-products and wastes for energy generation will be integrated into the whole process. Mass and energy balances, cost estimation and recommendations for technological design of the integrated process will be given.

1 Introduction

In view of the disuse of agricultural lands in Europe and other parts of the world, there is an ever increasing demand for alternative use options for agricultural products in the non-food sector. Renewable raw materials can be utilized directly, e.g. as energy carriers, as packaging materials, as fibres, for the production of colouring agents or as lubricants. However, they can also be converted biotechnologically by enzymes and microorganisms, giving us access to a multitude of new, biocompatible products and possible uses (MONTGOMERY 2004). Often the economy of bioprocesses is still the problem because in the case of bulk products the price is affected mainly by raw material costs (WILLKE & VORLOP 2004).

Biotechnological production of lactic acid as an example of these “building blocks” is carried out in technical reactors by using a suitable strain. Lactic acid, its salts and esters have a wide range of potential uses and are extensively used in diverse fields of food, industrial, cosmetic, pharmaceutical industries and agriculture (DATTA *et al.* 1995, KAMM *et al.* 1997 & 2000, DUDA & PENCZEK 2003). Besides the wide group of Lactobacilli (ANTONIO *et al.* 1996, HOFVENDAHL & HAHN-HÄGERDAHL 1997, BERRY *et al.* 1999, FU & MATHEWS 1999, KWON *et al.* 2001) other bacteria, e.g. Bacillus (PAYOT *et al.* 1999, DANNER *et al.* 2002, PATEL *et al.* 2004), and filamentous fungi (MARTAK *et al.* 2003), especially *Rhizopus oryzae* (YIN *et al.* 1997, BAI *et al.* 2003) were also used as production strains. The lactic acid bacteria convert easily monosaccharids like glucose or fructose into lactic acid, cell mass and byproducts depending on the species. This microbial synthesis is a multi-step reaction, the partial reactions of which are catalyzed by enzymes. The overall reaction equation can be written as



In this process, the cells grow and multiply forming biomass. Energy necessary for growth and maintenance is obtained by producing lactic acid. Lactic acid formation and cell growth are closely coupled in lactic acid fermentation. The microorganisms have a need for a carbon source, a source of nitrogen and nutrients, and a phosphorus source. The latter is available when inorganic phosphate salts are added to the medium. The demand for nitrogen can't be covered by inorganic salts only. Lactic acid bacteria need also a series of nitrogen-containing nutrients (amino acids, peptides etc.) for growth and therefore, the medium have to be supplied by complex protein hydrolyzates (yeast extract, peptone etc.). The protein extracts mentioned are very expensive and their substitution by low-priced nutrient extracts is necessary when a large-scale production is planned. A useful combination of green biomass processing for the production of fodder pellets and the utili-

zation of the pressed juice for the lactic acid fermentation was described by (ANDERSEN & KIEL 2000). The use of date juice together with different nitrogen sources as a substrate for lactic acid production was investigated by (NANCIB *et al.* 2001).

In recent years, new lactic acid production processes consisting of the operations of enzymatic hydrolysis of starch (GROHMANN & BOTHAST 1997, ANURADHA *et al.* 1999; MIURA *et al.* 2003; FUKUSHIMA *et al.* 2004) and cellulose (MELZUCH *et al.* 1997, MOLDES 1999, WOICIECHOWSKI 1999, PARK *et al.* 2004, WEE *et al.* 2004), fermentation process (RICHTER & BEYER 1998, PAYOT *et al.* 1999, KWON *et al.* 2001, BULUT *et al.* 2004, BUSTOS *et al.* 2004, TANIGUCHI *et al.* 2004), lactic acid separation and product purification (SIEBOLD *et al.* 1995, FRIELING & SCHÜGERL 1999, CAO *et al.* 2002, BAILLY 2002, HUANG *et al.* 2004, BOUCHOUX *et al.* 2005) were developed.

The research is advancing focused on the use of renewable agricultural raw materials as carbon substrates as well as protein sources. In this context, there is an strong interest to reduce costs for raw materials and to use renewable resources like grain and green biomass respectively. In this research, the objective was to test the suitability of grains (barley, rye) and green masses (lupine, lucerne) for use as raw materials in lactic acid fermentation.

Due to the importance of separation and purification of the product the investigations concentrate on the down-stream processes of lactic acid in the form of sodium lactate made by fermentation of the renewable raw material barley. Conventional processes are based on precipitation steps that generate large amounts of chemical effluents (BAILEY & OLLIS 1986). Consequently the environmental impact and the operating costs of traditional precipitation processes can be reduced by using alternative technologies, such as two-stage electrodialysis (GLASSNER & DATTA 1990, LEE *et al.* 1998). In the first step of desalting electrodialysis, sodium lactate is recovered, purified and concentrated. In the water-splitting or acidification step, lactic acid is generated from sodium lactate, and sodium hydroxide is recovered and purified (KIM & MOON 2001). Membrane operations, for instance microfiltration, nanofiltration, and reverse osmosis, as well as monopolar (EDM) and bipolar (EDB) electrodialysis, are increasingly being used as an alternative to conventional methods such as filtering, separating, crystallizing, vaporization, drying, and ion exchange (SIRKA *et al.* 1999). The down-stream processes were performed as reported by (BAILLY 2002) for the production of fermented organic acids, based on membrane processes and a final purification stage using ion exchange resins. The efficiency of each down stream process is stated.

2 Strain selection and strain characterization

One of the objectives stated in the project BESUB is a new process for producing lactic acid, which can be used in a biorefinery. In this context, there is a need to find a high-productivity bacterium strain. This strain will be the basis for developing a batch and continuous fermentation technology respectively.

2.1 Objectives

The aim of this research was to identify a lactic acid bacteria strain, which is suitable for producing lactic acid from barley in a technical facility. This strain should be characterized by

- a high lactic acid producing capacity,
- a high selectivity for producing pure L(+)-lactic acid and
- a highly stability.

It should also not be patented for use by other individuals/ companies.

To solve this problem, the following tests had to be carried out:

- screening in shake flask experiments and selection of strains for further testing
- preliminary assessment of the fermentation performance of the strains chosen in discontinuous fermenter experiments and selection of the best strain
- phenotypic optimisation of the selected strain
- determining the kinetic data of cell growth and product formation.

2.2 Screening program

A series of strains of the species *Bacillus*, *Lactobacillus* and *Pediococcus*, which had not previously been used in any fermentation process, were purchased from the German Collection of Microorganisms and Cell Cultures Braunschweig and partly chosen from the strain collection of the ATB Potsdam, respectively. The acidifying power of the microorganisms selected was firstly tested in shaking flask experiments. To achieve this, the bacteria were cultivated discontinuously in a special medium without pH control and the pH value was measured after 24 h and 48 h (**Figure 2**). According to the thermal growth behaviour of the microorganisms used, the temperature for this screening step varied between 28°C and 40°C.

The pH value decreases during the fermentation due to the formation of lactic acid. Therefore, the decrease of the pH value can be considered as a measure of the lactic acid production. The deeper the pH value drops during the fermentation the more the amount of lactic acid in the medium increases. Significant decreasing of pH below a value of 4.0 gives also evidence for the stability of the strain tested.

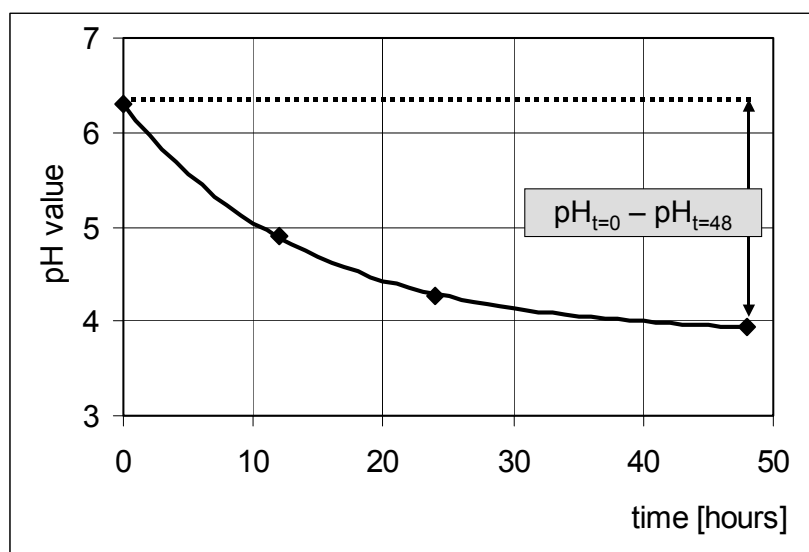


Figure 2: Decrease of pH during discontinuous fermentation

Selected results of this research are shown in **Table 1**. The best results (absolute decrease of $\Delta\text{pH} \geq 1.3$ and minimum pH attained < 3.8) were obtained with the three strains *Lactobacillus paracasei* 168, *Lactobacillus paracasei* 169 and *Lactobacillus paracasei* 172. All of the other strains tested were unable to decrease the pH below a value of 3.8. Therefore these three strains were chosen for subsequent optimisation.

Table 1: Results of the strain screening by measuring the decrease of pH in standard medium MRS

No. in ATB Collection	Species	Lactate	Temp.	pH-value after time t			$\text{pH}_{t=0} - \text{pH}_{t=48}$
				t = 0	t = 24h	t = 48h	
158	<i>Bacillus coagulans</i>	L(+)	40°C	5.40	4.64	4.39	1.01
161	<i>Bacillus coagulans</i>	L(+)	37°C	5.53	5.50	5.45	0.08
162	<i>Bacillus coagulans</i>	L(+)	30°C	5.60	5.57	5.47	0.13
167	<i>Bacillus coagulans</i>	L(+)	37°C	5.34	4.70	4.47	0.87
168	<i>Lb. paracasei</i> , subspec. <i>paracasei</i>	L(+)	30°C	4.92	3.75	3.62	1.30
169	<i>Lb. paracasei</i> , sub- spec. <i>paracasei</i>	L(+)	30°C	5.02	3.83	3.62	1.40
171	<i>Pediococcus dextrini-</i> <i>cus</i>	L(+)	30°C	5.21	4.52	4.28	0.93
172	<i>Lb. paracasei</i> , sub- spec. <i>paracasei</i>	L(+)	30°C	5.02	3.91	3.66	1.36
173	<i>Pediococcus dextrini-</i> <i>cus</i>	L(+)	30°C	5.12	4.13	3.9	1.22
179	<i>Lb. sakei</i>	L(+)	30°C	5.22	4.14	3.96	1.26
182	<i>Lb. sharpeae</i>	L(+)	30°C	5.53	5.5	5.52	0.01
186	<i>Lb. ruminis</i>	D/L	37°C	5.49	5.28	5.28	0.21
187	<i>Lb. amylophilus</i>	L(+)	28°C	5.53	5.29	4.77	0.76
191	<i>Lb. animalis</i>	L(+)	37°C	5.15	4.31	4.15	1.00

2.3 Preliminary assessment of fermentation performance

This investigation was carried out in a laboratory stirred tank (vessel volume 3 litres) bioreactor BIostat MD (B. Braun Biotech International GmbH, Germany) equipped with a digital control unit DCU. The pH value was controlled at the respective set point, using NaOH as a correction agent. The medium used was a modified model medium (MRS) containing glucose as carbon source ($40 \text{ g} \cdot \text{L}^{-1}$ and $100 \text{ g} \cdot \text{L}^{-1}$) and meat extract and peptone ($10 \text{ g} \cdot \text{L}^{-1}$) as nitrogen sources. The time course of lactic acid production was observed. The results are given in **Figure 3 - 5**.

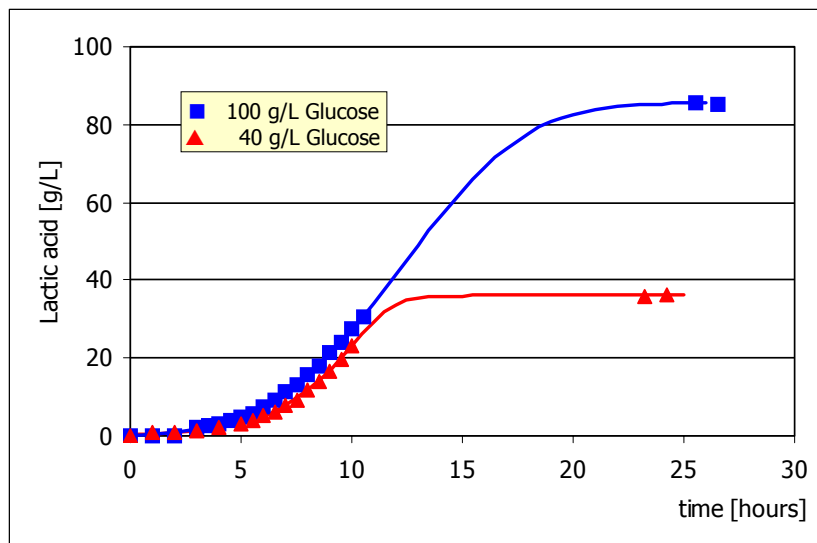


Figure 3: Time course of lactic acid formation in discontinuous cultivation of the strain *Lactobacillus paracasei* 168 on model media containing glucose in quantities of $40 \text{ g} \cdot \text{L}^{-1}$ and $100 \text{ g} \cdot \text{L}^{-1}$

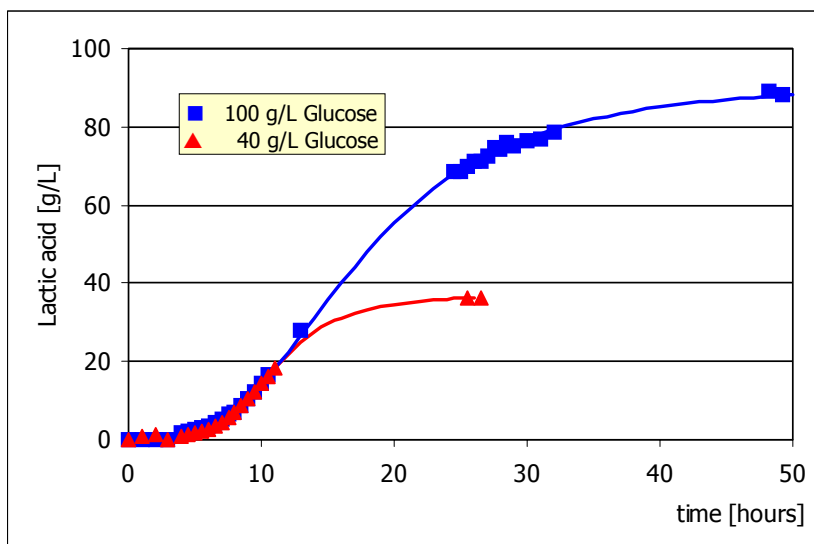


Figure 4: Time course of lactic acid formation in discontinuous cultivation of the strain *Lactobacillus paracasei* 169 on model media containing glucose in quantities of $40 \text{ g} \cdot \text{L}^{-1}$ and $100 \text{ g} \cdot \text{L}^{-1}$

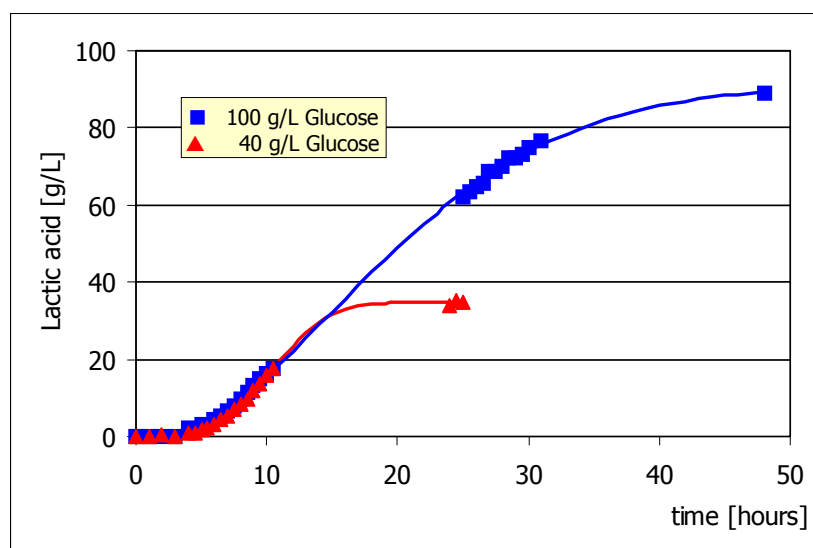


Figure 5: Time course of lactic acid formation in discontinuous cultivation of the strain *Lactobacillus paracasei* 172 on model media containing glucose in quantities of $40 \text{ g} \cdot \text{L}^{-1}$ and $100 \text{ g} \cdot \text{L}^{-1}$

The three strains are able to use the substrate completely when the glucose concentration is at $100 \text{ g} \cdot \text{L}^{-1}$. The lactic acid yields reach 88 % to 90 % in this case. Comparing the curves of the three strains, it is noticeable that the strain *L. paracasei* 168 shows the greatest slope in lactic acid accumulation. Therefore, it can be concluded, that this strain is the most efficient one. This is also made clear by the fact that the cultivation of the strain *L. paracasei* 168 on a medium initially containing $100 \text{ g} \cdot \text{L}^{-1}$ of glucose is terminated in a much shorter time than is possible when the other two strains are used (**Table 2**).

Table 2: Duration of batch cultivation observed with 3 selected strains on a medium with an initial glucose content of $100 \text{ g} \cdot \text{L}^{-1}$

Strain	Duration of fermentation (h)	Lactate yield (%)
<i>Lactobacillus paracasei</i> 168	26	90
<i>Lactobacillus paracasei</i> 169	43	88
<i>Lactobacillus paracasei</i> 172	50	88

2.4 Phenotypic strain optimisation

The strain *Lactobacillus paracasei* 168 was selected to be the most appropriate by means of the methods described above. In this section, a methodology is shown for optimising the environmental parameters of temperature and pH value with respect to both the lactic acid production and cell growth of this strain.

2.4.1 Methods

The methods used were an experimental design-aided trial methodology, an approximation of model functions and a surface response method of parameter optimisation.

2.4.1.1 Experimental design

The optimum values of some process parameters for cell growth and lactic acid production were determined in discontinuous fermentation experiments with pH control in a 5-L-fermentor using a model MRS medium and an extended 2^3 factorial experimental design. Temperature and pH value, each set up at three and four levels respectively, were the independent variables and the specific growth rate, specific lactate formation rate, the duration of fermentation and the lactic acid yield were the dependent variables:

Independent variables:

Temperature (T):	28°C	33°C	38°C	40.5°C	-
pH value (pH):	5.5	6.0	6.5	-	7.0
trial code of variables	-1	0	+1	+1.5	+2

The experimental scheme containing 15 trials is shown in **Table 3** and **Figure 6**.

Each point in the three-dimensional scheme (**Figure 5**) represents a trial with one of the parameter combinations defined in **Table 3**.

Table 3: Experimental scheme of phenotypic strain optimisation

T	pH
+1.5	-1
+1.5	0
+1.5	+1
+1	-1
+1	0
+1	+1
+1	+2
0	-1
0	0
0	+1
0	+2
-1	-1
-1	0
-1	+1
-1	+2

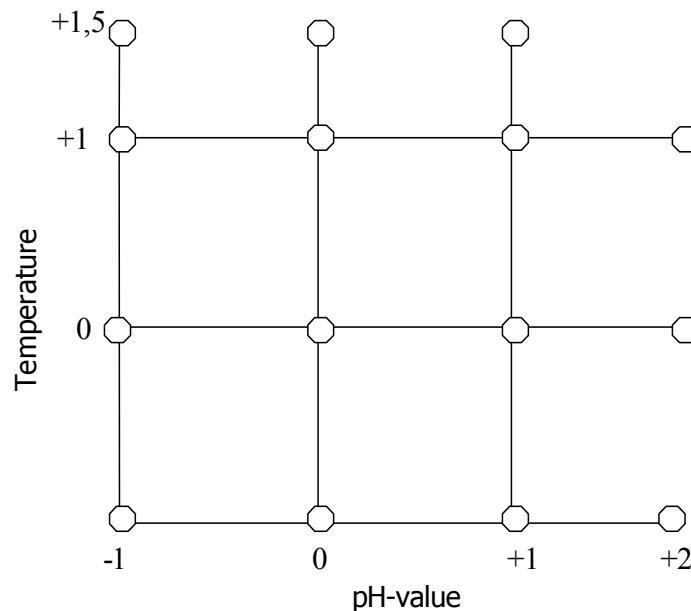


Fig. 6: Three-dimensional diagram of experiments

2.4.1.2 Conditions of cultivation

Batch cultivations were carried out in a stirred tank reactor under the condition of both temperature and pH value control (**Figure 7**).

The medium used was a modified MRS (created by de Man, Rogosa and Sharpe, 1960) medium. It contained 120 g of glucose, 5 g of yeast extract, 10 g of peptone, 10 g of meat extract, 1 g of Tween 80, 5 g of sodium acetate, 2 g of ammonium citrate, 2 g of K_2HPO_4 , 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.05 g of $MnSO_4 \cdot H_2O$ and 1 g of NH_4Cl per litre. The volume of inoculum amounted to 180 mL in each of the experiments carried out.



Figure 7: BIOSTAT MD (B. Braun Biotech International GmbH, Germany) equipped with a digital control unit DCU

2.4.1.3 Test description

Experiments were started after the process parameters (temperature, pH value) were fixed at their desired level and the inoculum was added. Samples were taken from the medium during the experiments at time intervals of 2 hours in order to determine the actual concentrations of glucose, lactic acid and cell mass in the fermentation broth. The trials were terminated when the glucose was exhausted.

2.4.1.4 Evaluation of experiments

The kinetic data needed for characterizing a strain can be derived from the concentrations of reactants estimated during the fermentation at different times. In **Table 4** the measured values of lactic acid, glucose and biomass (the latter as dry matter) are listed for a batch culture of *Lactobacillus paracasei* 168 on a $120 \text{ g} \cdot \text{L}^{-1}$ glucose medium.

Table 4: Concentrations of reactants in batch fermentation with the strain *L. paracasei* 168 (initial glucose concentration: 120 g · L⁻¹, T: 28°C; pH: 5.5)

Time t (h)	Lactic acid P (g · L ⁻¹)	Glucose S (g · L ⁻¹)	Biomass X (g · L ⁻¹)
0	0	115.77	0
2	0.88	110.34	0.42
4	1.93	109.38	0.39
6	3.83	108.28	0.42
8	7.39	106.27	1.62
26	65.87	42.20	8.44
28	70.59	37.15	8.22
30	76.33	33.05	8.56
32	80.06	28.82	8.67
49.25	104.56	2.49	8.08
51.25	106.28	0	8.43
54	107.19	0	8.65

Initially, time functions describing exactly the change of the concentrations of glucose, lactic acid and biomass are determined by model approximation. In these cases, the sigmoidal equation (equation 1) was found to be suitable for use as a target function. This procedure could be easily achieved by means of the computer programme TableCurve 2D (Jandel Scientific Software).

$$P_{(t)}, X_{(t)}, S_{(t)} = a + \frac{b}{(1 + \exp(-(t - c) / d))} \quad (1)$$

The factors a, b, c, and d are constants which are calculated by the computer by iteration. The time functions obtained on the way described are presented in **Figure 8 - 10**. The accuracy data displayed at the top of each diagram supports the excellent model approximation attained using the sigmoidal equation.

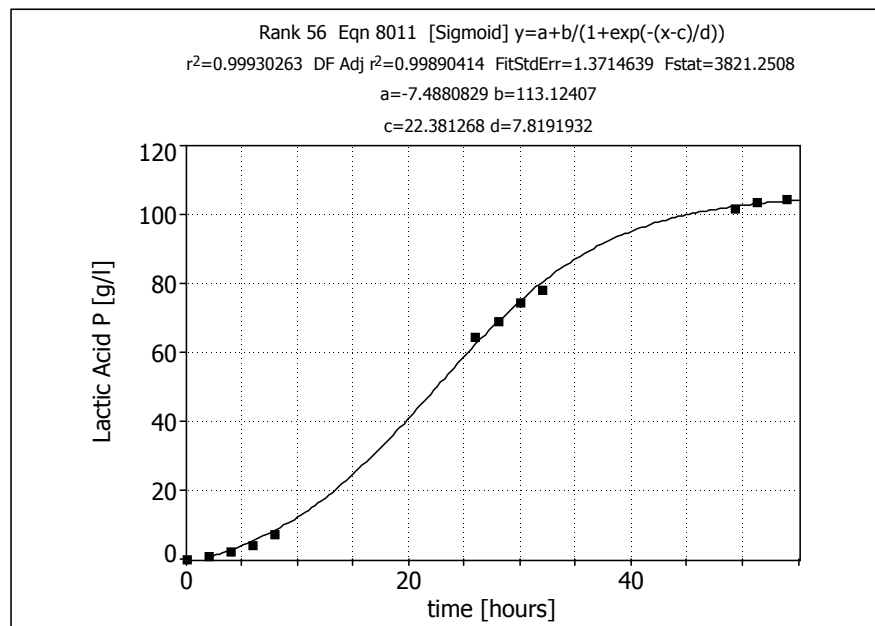


Figure 8: Time function of lactic acid concentration in a batch cultivation of *L. paracasei* 168 (Initial glucose concentration $110 \text{ g} \cdot \text{L}^{-1}$; Temp: 28°C ; pH: 5.5)

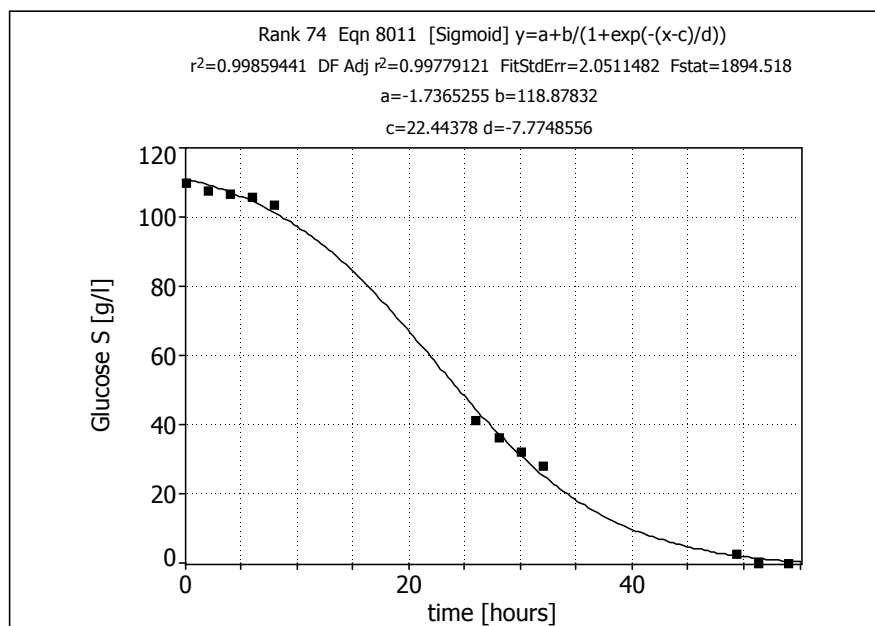


Figure 9: Time function of glucose concentration in a batch cultivation of *L. paracasei* 168 (Initial glucose concentration $110 \text{ g} \cdot \text{L}^{-1}$; Temp: 28°C ; pH: 5.5)

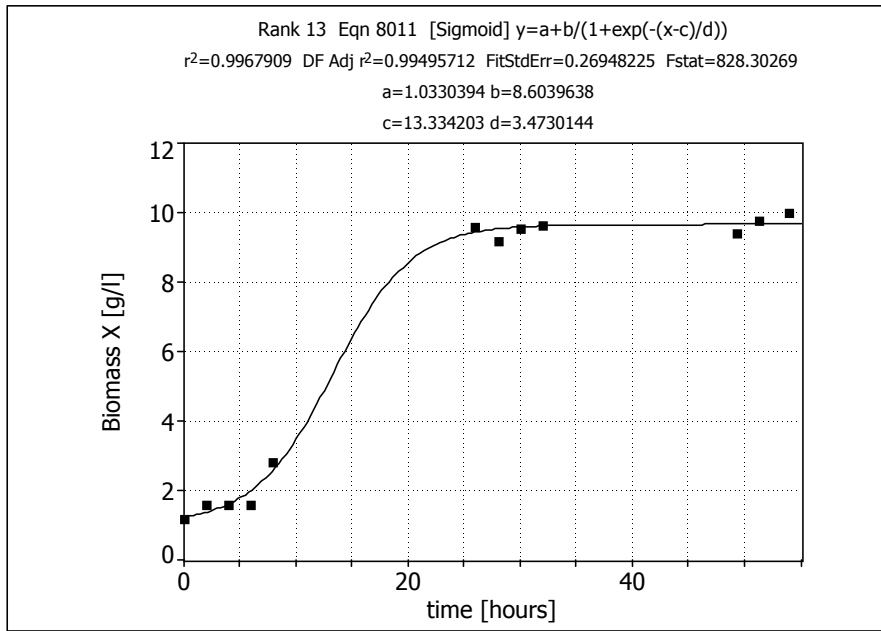


Figure 10: Time function of biomass concentration in a batch cultivation of *L. paracasei* 168 (Initial glucose concentration $110 \text{ g} \cdot \text{L}^{-1}$; Temp: 28°C ; pH: 5.5)

The second stage is to form the first derivation of equation (1):

$$\frac{dP}{dt}, -\frac{dS}{dt}, \frac{dX}{dt} = \frac{\frac{b}{d} \cdot \exp(-(t-c)/d)}{(1 + \exp(-(t-c)/d))^2} \quad (2)$$

and obtain through this the time course of the volumetric production rates and the glucose degradation rate. All of these curves run through maxima representing the maximal productivities of growth, lactate production or the maximal glucose degradation rates, which may be achieved by the strain tested under the conditions of cultivation tested (**Figure 11**).

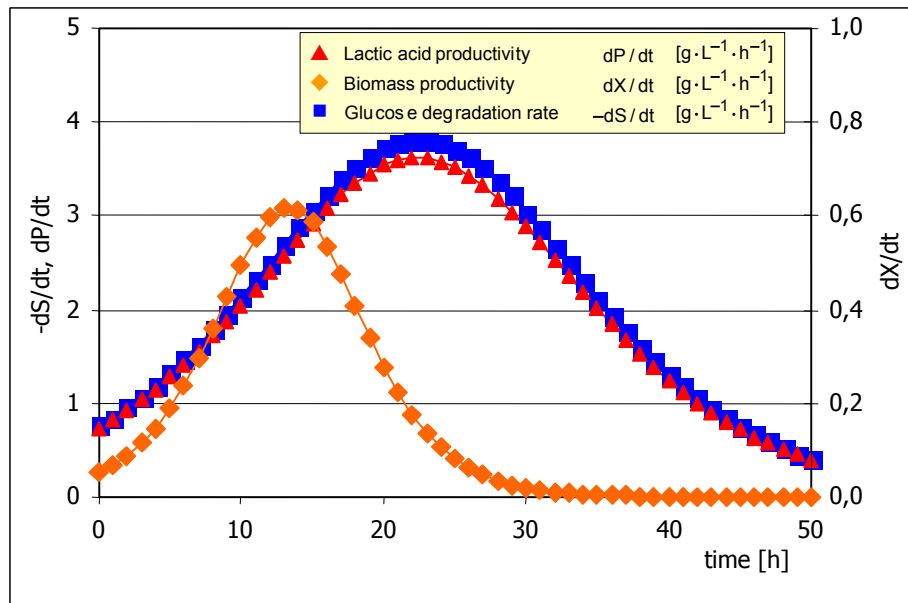


Figure 11: Time functions of lactic acid and biomass productivity as well as volumetric glucose degradation rate in a batch cultivation of *L. paracasei* 168 (Initial glucose concentration $110 \text{ g} \cdot \text{L}^{-1}$; Temp: 28°C ; pH: 5.5)

Thirdly, we can obtain the time course function of the specific capacity of a strain by dividing equation (2) by the time course function of cell concentration. When using equation (2) for lactic acid production and cell mass formation, the time function of the specific lactate formation rate (equation 3) and the specific growth rate (equation 4), is obtained. In an analogous way we obtain the time function of the specific glucose degradation rate (equation 5).

$$\left[\frac{\frac{b}{d} \cdot \exp(-(t-c)/d)}{(1 + \exp(-(t-c)/d))^2} \right]_{\text{lactate}} \cdot \left[\frac{1}{a + \frac{b}{(1 + \exp(-(t-c)/d))}} \right]_{\text{biomass}} = \pi = \frac{1}{X} \cdot \frac{dP}{dt} \quad (3)$$

$$\left[\frac{\frac{b}{d} \cdot \exp(-(t-c)/d)}{(1 + \exp(-(t-c)/d))^2} \right]_{\text{biomass}} \cdot \left[\frac{1}{a + \frac{b}{(1 + \exp(-(t-c)/d))}} \right]_{\text{biomass}} = \mu = \frac{1}{X} \cdot \frac{dX}{dt} \quad (4)$$

$$\left[\frac{\frac{b}{d} \cdot \exp(-(t-c)/d)}{(1 + \exp(-(t-c)/d))^2} \right]_{\text{glucose}} \cdot \left[\frac{1}{a + \frac{b}{(1 + \exp(-(t-c)/d))}} \right]_{\text{biomass}} = \sigma = \frac{1}{X} \cdot \frac{(-dS)}{dt} \quad (5)$$

The time function of the specific lactic acid formation rate π according to the equation (3) is plotted in **Figure 12**. The points marked are characteristic performance data of the strain, which can be used as criteria for strain optimization.

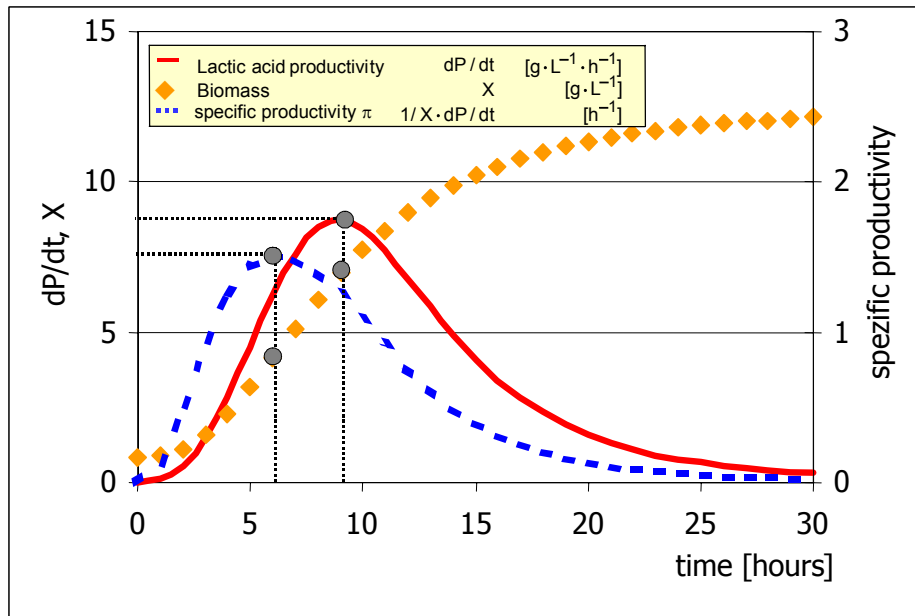


Figure 12: Courses of the time functions of the lactic acid productivity, the specific lactate formation rate and the biomass concentration during a typical batch fermentation of the strain *L. paracasei* 168

2.4.2 Results

The objective of these batch experiments was to calculate quantities that are characteristic of the strain *Lactobacillus paracasei* 168 and suitable for optimising process parameters. For this purpose, time-dependent concentrations of lactic acid, biomass and glucose measured during the trials were used as starting data. Some of the strain-characteristic quantities could be related directly from the latter, some other quantities had to be estimated by fitting target functions on the time-dependent data and calculating the turning points of these functions.

2.4.2.1 Characteristic quantities related directly from the measured data

This is in reference to the final concentrations of lactic acid and biomass, the duration of fermentation and the yields of lactic acid and biomass. The values found are listed in **Table 5**.

Table 5: Measuring data and characteristic performance data (*Lactobacillus paracasei* 168)

T(°C)	pH	X _{max}	P _{max}	Y _P	Y _x	Δt
40.5	5.5	10.7	104.8	88.1	8.99	40
40.5	6.0	10.75	103.3	93.5	9.73	31.5
40.5	6.5	12.2	104.2	92.5	10.8	26.0
38	5.5	10.6	105.0	83.9	8.5	34.25
38	6.0	11.8	102.5	89.7	10.3	23.4
38	6.5	13.1	99.15	80.9	10.7	27.5
38	7.0	8.2	103.3	97.6	7.75	43.1
33	5.5	26.8	102.8	92.3	25.1	44.2
33	6.0	14.2	102.0	91.3	12.8	26.4
33	6.5	12.3	102.8	93.5	11.2	26.7
33	7.0	9.1	102.9	94.4	8.35	46.5
28	5.5	8.65	107.2	92.5	7.47	54
28	6.0	15.2	104.3	91.9	13.4	30.7
28	6.5	19.8	102.8	89.4	17.2	30.4
28	7.0	9.8	101.1	91.3	8.86	45.6

X _{max}	Final biomass concentration in batch fermentation	g · L ⁻¹
P _{max}	Final lactic acid concentration in batch fermentation	g · L ⁻¹
Y _P	Mean yield of lactic acid	%
Y _x	Mean yield of biomass	%
Δt	Duration of fermentation	h

Table 5 shows that the new strain is able to accumulate more than 100 g of lactic acid per litre in the medium. The yield of lactic acid can be higher than 90 % and the duration of fermentation varies between 23 and 54 hours.

2.4.2.2 Estimate of kinetic values for product formation and cell growth from experimental data by model approximation

In this context, the maximum productivities of both lactic acid and biomass production, the maximum glucose utilization rate and the corresponding specific rates of the formation of lactic acid and biomass as well as the specific glucose degradation rate are the most important quantities. The values obtained are shown in **Table 6**.

Table 6: Measuring data and kinetic data obtained by means of model approximation

T (°C)	pH	μ_1	μ_2	μ_{\max}	π_1	π_2	π_{\max}	$-\sigma_1$	$-\sigma_2$	$-\sigma_{\max}$	(dX/dt) ax.	(dP/dt) max.	-(dS/dt) max.
40.5	5.5	-	0.52	-	2.70	0.81	-	4.46	1.40	-	0.59	4.22	5.30
40.5	6.0	0.75	0.31	-	1.31	1.54	1.61	1.60	1.59	1.68	0.81	10.71	10.95
40.5	6.5	0.52	0.21	-	3.0	1.84	-	3.36	1.89	-	1.13	10.89	11.49
38.0	5.5	0.52	0.23	-	2.81	0.58	-	3.63	0.67	-	0.89	4.41	5.04
38.0	6.0	0.38	0.18	-	2.29	1.77	2.33	2.37	1.99	2.52	1.04	11.08	12.64
38.0	6.5	0.39	0.17	-	1.17	1.49	1.67	2.10	1.73	2.9	0.92	10.48	11.33
38.0	7.0	0.42	0.17	-	2.80	1.01	-	2.80	0.96	-	0.56	5.79	5.60
33.0	5.5	0.42	0.19	-	2.39	0.46	-	2.51	0.51	-	0.76	3.76	4.21
33.0	6.0	0.46	0.18	-	1.65	1.13	-	1.9	1.22	-	1.28	10.0	10.72
33.0	6.5	0.34	0.14	-	1.68	1.58	1.93	1.15	2.09	2.34	0.83	9.86	12.72
33.0	7.0	0.45	0.23	-	1.71	0.5	-	1.83	0.53	-	0.94	4.15	4.39
28.0	5.5	0.13	0.12	0.16	1.82	0.59	1.88	1.76	0.62	1.76	0.53	3.72	4.02
28.0	6.0	0.34	0.09	-	1.33	0.89	-	1.08	1.04	1.18	0.64	6.34	7.98
28.0	6.5	0.36	0.08	-	1.21	0.95	1.17	1.19	1.03	-	0.88	7.38	8.52
28.0	7.0	0.37	0.21	0.38	1.63	0.59	-	1.75	0.69	-	0.96	5.05	5.53

$(dX/dt)_{\max}$	Maximum value of the biomass productivity during batch fermentation	$g \cdot L^{-1} \cdot h^{-1}$
$(dP/dt)_{\max}$	Maximum value of the lactic acid productivity during batch fermentation	$g \cdot L^{-1} \cdot h^{-1}$
$(-dS/dt)_{\max}$	Maximum value of glucose utilisation rate during batch fermentation	$g \cdot L^{-1} \cdot h^{-1}$
μ_{\max}	Maximum value of the specific growth rate during batch fermentation	h^{-1}
μ_1	Value of specific growth rate when it is identical with that of biomass productivity	h^{-1}
μ_2	Value of specific growth rate at the moment of maximum biomass productivity	h^{-1}
π_{\max}	Maximum value of the specific lactic acid formation rate during batch fermentation	h^{-1}
σ_{\max}	Maximum value of the specific glucose degradation rate during batch fermentation	h^{-1}
π_1	Value of specific lactic acid formation rate when it is identical with that of lactic acid productivity	h^{-1}
$\pi_2, \sigma_1, \sigma_2$	accordingly to the previous specific reaction rates	h^{-1}

It may be stated that the kinetic quantities had a wide range of variance depending on the actual values of temperature and pH. The maximum lactic acid productivity and the maximum biomass productivity can be between 3.7 and 11 [$g \cdot L^{-1} \cdot h^{-1}$] and 0.5 and 1.3 [$g \cdot L^{-1} \cdot h^{-1}$], respectively. In this way, the maximum values of the specific growth rate, the specific product production and the specific glucose utilisation rate were found to be $\mu = 0.75 h^{-1}$, $\pi = 3.00 h^{-1}$, $\sigma = 4.46 h^{-1}$.

2.4.2.3 Estimation of the optimal parameters for lactic acid production and cell growth by means of non-linear regression

The data matrices shown in **Table 5** and **Table 6** were used in parameter optimisation by non-linear and three-dimensional regression using the computer program TableCurve 3D v2 (Jandel Scientific Software).

The duration of fermentation is the time interval between starting the process by inoculation and break off when glucose is exhausted completely. For its dependence on temperature and pH value a model equation of the type

$$\Delta t = a + b \cdot T + \frac{c}{pH} + d \cdot T^2 + \frac{e}{pH^2} + \frac{f \cdot T}{pH} + g \cdot T^3 + \frac{h}{pH^3} + \frac{i \cdot T}{pH^2} + \frac{j \cdot T^2}{pH} \quad (6)$$

was derived ($r^2 = 0.98319$). Its constants were found to be

$a = 6641.0714$, $b = 47.62301$, $c = -123641.65$, $d = -2.7939018$, $e = 655138.79$,
 $f = 544.85337$, $g = 0.022021249$, $h = -1055964.1$, $i = -2584.6406$, $j = 4.0491776$.

The model describes a central minimum (**Figure 13**) at the coordinates $T = 37^\circ\text{C}$ and $pH = 6.2$. The shortest duration of fermentation amounts to 21.8 hours.

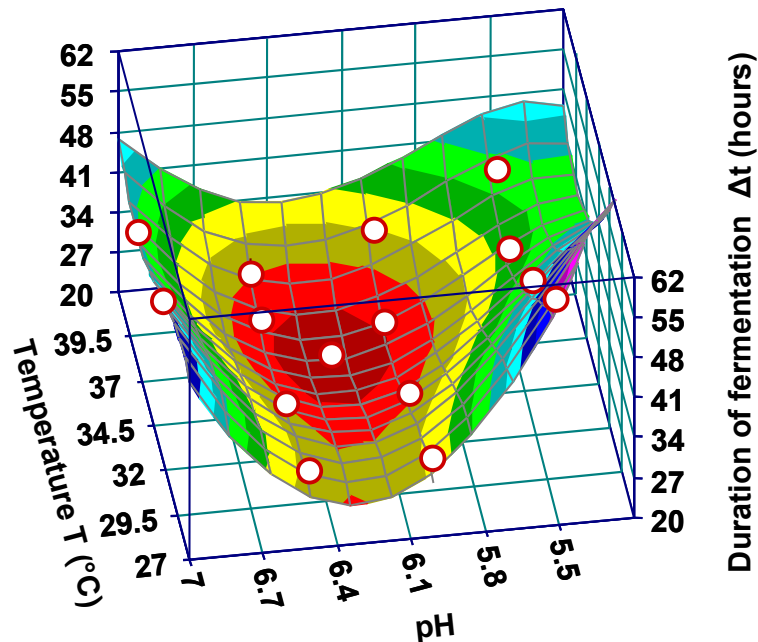


Figure 13: Spherical diagram of the function $\Delta t = f(T, pH)$: side view

The final lactic acid concentration is the lactic acid concentration at the moment of break off. It is the maximum lactic acid concentration, which can be realized by the strain under the respective process conditions. Using the corresponding values from **Table 6** the model approximation ($r^2 = 0.9929$) results in a model equation having the structure of equation (7).

$$P_{final} = a + \frac{b}{T} + \frac{c}{pH} + \frac{d}{T^2} + \frac{e}{pH^2} + \frac{f}{T \cdot pH} + \frac{g}{T^3} + \frac{h}{pH^3} + \frac{i}{T \cdot pH^2} + \frac{j}{T^2 \cdot pH} \quad (7)$$

Its constants are

$A = -610.01731$, $b = 4952.1035$, $c = 13174.471$, $d = 115013.77$, $e = -71912.831$,
 $f = -131679.11$, $g = -6223001.8$, $h = 158215.24$, $i = -122891.92$, $j = 3054063.2$.

A diagram of this model is illustrated in **Figure 14**.

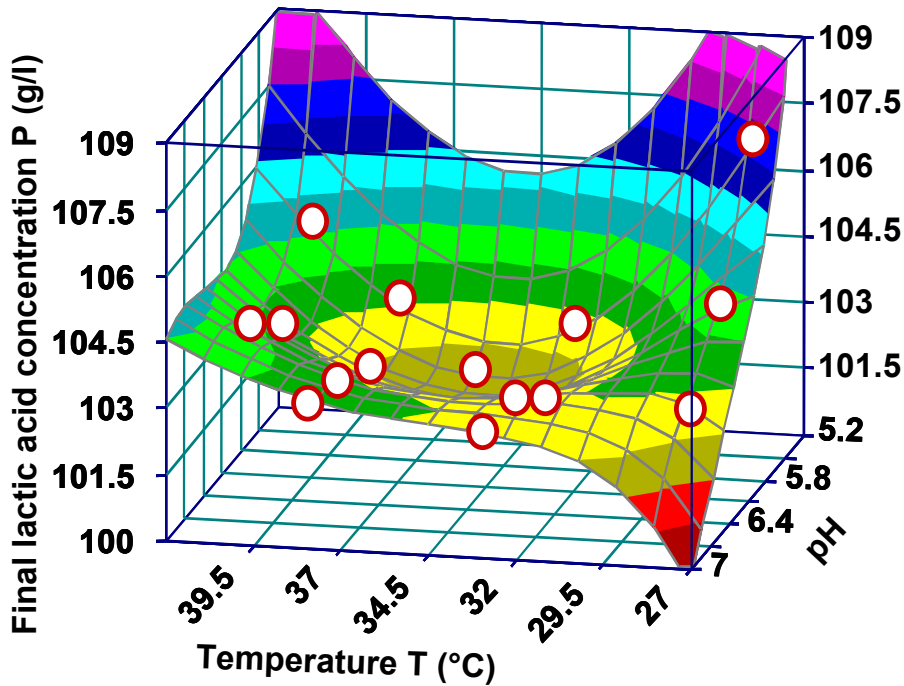


Figure 14: Spherical diagram of the function $P_{final} = f(T, pH)$

Unfortunately, this model shows a minimum as optimum value and so there is no sole parameter combination, which guarantees a maximum lactic acid concentration. The minimum final concentration amounts to $101.6 \text{ g} \cdot \text{L}^{-1}$ and is realized at a temperature of $T = 35^\circ\text{C}$ and a pH value of 5.8. But it is clear that there are other parameter combinations for higher final lactic acid concentrations up to $107 \text{ g} \cdot \text{L}^{-1}$.

The mean yield of lactic acid is the overall lactic acid yield calculated by dividing the final amount of lactic acid by the amount of glucose utilized. The model of mean lactic acid yield has a mathematical structure (equation (8)) like that found for the duration of fermentation, but it shows a distinct minimum and maximum (**Figure 15**).

$$Y_p = a + b \cdot T + \frac{c}{pH} + d \cdot T^2 + \frac{e}{pH^2} + \frac{f \cdot T}{pH} + g \cdot T^3 + \frac{h}{pH^3} + \frac{i \cdot T}{pH^2} + \frac{j \cdot T^2}{pH} \quad (8)$$

The constants of equation (8) are:

$a = 5610.4949$, $b = 4.0826278$, $c = -101847.05$, $d = -0.94274624$, $e = 584453.27$,
 $f = 338.79754$, $g = 0.013115906$, $h = -1126333.1$, $i = -694.84883$, $j = -2.0450459$
 $(r^2 = 0.93502)$.

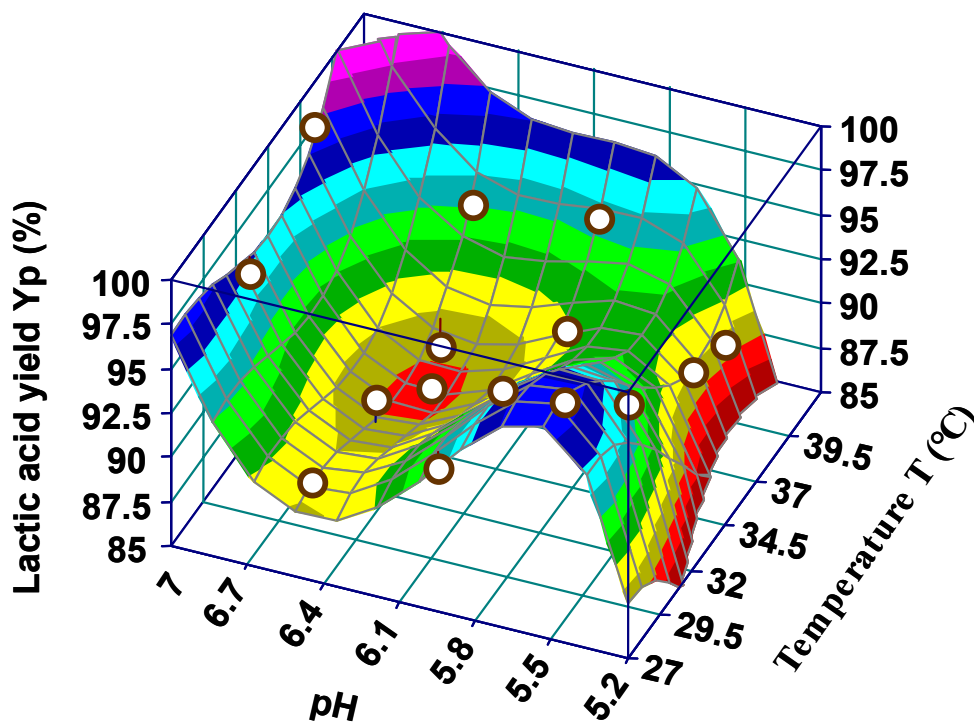


Figure 15: Spherical diagram of the function $Y_p = f(T, pH)$

The minimum lactic acid yield (87.1 %) is situated within the parameter limits chosen in this research. Its coordinates are $T = 35^\circ\text{C}$ and $pH = 6.45$. A maximum of 97.1 % was found at $T = 28.5^\circ\text{C}$ and $pH = 5.58$.

The calculated values of lactic acid productivity can be described as a function of the independent variables temperature and pH. A very good approximation of a polynomial function of the type

$$\frac{dP}{dt} = a + \frac{b}{T} + \frac{c}{pH} + \frac{d}{T^2} + \frac{e}{pH^2} + \frac{f}{T \cdot pH} + \frac{g}{T^3} + \frac{h}{pH^3} + \frac{i}{T \cdot pH^2} + \frac{j}{T^2 \cdot pH} \quad (9)$$

on the values of lactic acid productivity was achieved ($r^2 = 0.9829$). A three-dimensional presentation of this function is shown in **Figure 16**.

The constants of equation (9) were found to be

$a = -1897.8994$, $b = 38365.077$, $c = 26087.906$, $d = -572939.72$, $e = -122441.37$,
 $f = -261910.42$, $g = 6274659.1$, $h = 170643.38$, $i = 822647.81$, $j = -67681.892$.

There is a distinct productivity maximum of $11.8 \text{ g} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ at the parameters $T = 39^\circ\text{C}$ and $\text{pH} = 6.25$.

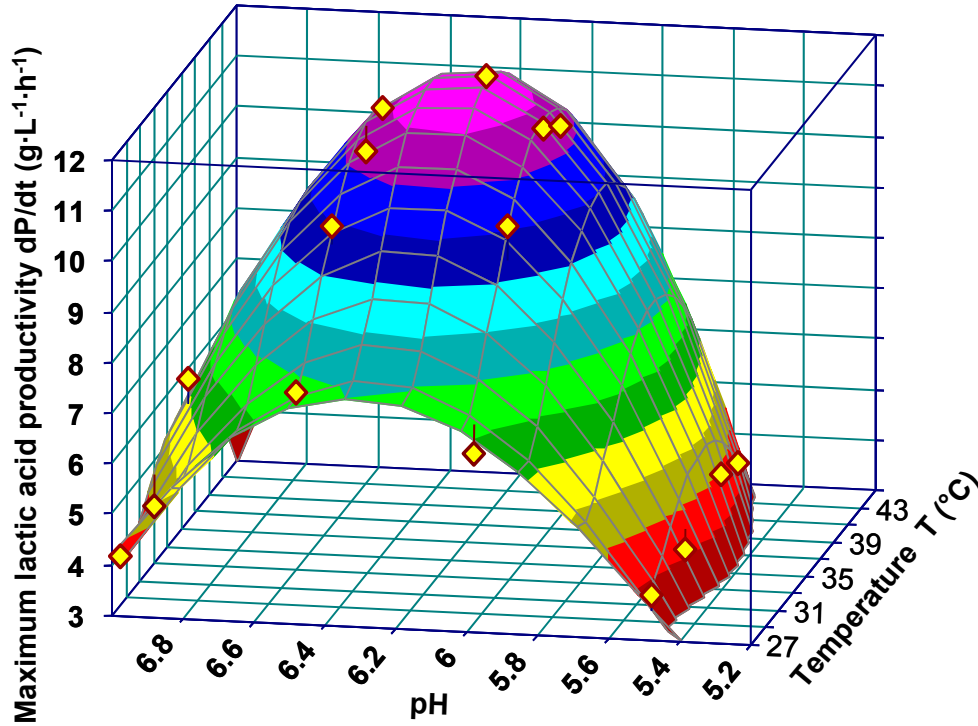


Figure 16: Spherical diagram of the function $dP/dt = f(T, \text{pH})$

For the function $dX/dt = f(T, \text{pH})$, a good model approximation (**Figure 17**) was attained by using the following target equation

$$\left[\frac{dX}{dt} \right]_{\max} = a + \frac{b}{T} + \frac{c}{\text{pH}} + \frac{d}{T^2} + \frac{e}{\text{pH}^2} + \frac{f}{T \cdot \text{pH}} + \frac{g}{T^3} + \frac{h}{\text{pH}^3} + \frac{i}{T \cdot \text{pH}^2} + \frac{j}{T^2 \cdot \text{pH}} \quad (10)$$

with

$a = -220.60104$, $b = -3162.5331$, $c = 4478.8015$, $d = 202051.44$, $e = -23988.084$,
 $f = -30186.595$, $g = -2285413$, $h = 41609.47$, $i = 96804.513$, $j = -40039.057$
 $(r^2 = 0.9015)$.

This function also forms a maximum. It has a value of $1.3 \text{ g} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ and its parameter coordinates are not identical with those of lactic acid productivity. The optimum temperature for growth productivity amounts to 31.4°C and is significantly lower than the optimum temperature for lactic acid production. The optimum pH value is 6.52.

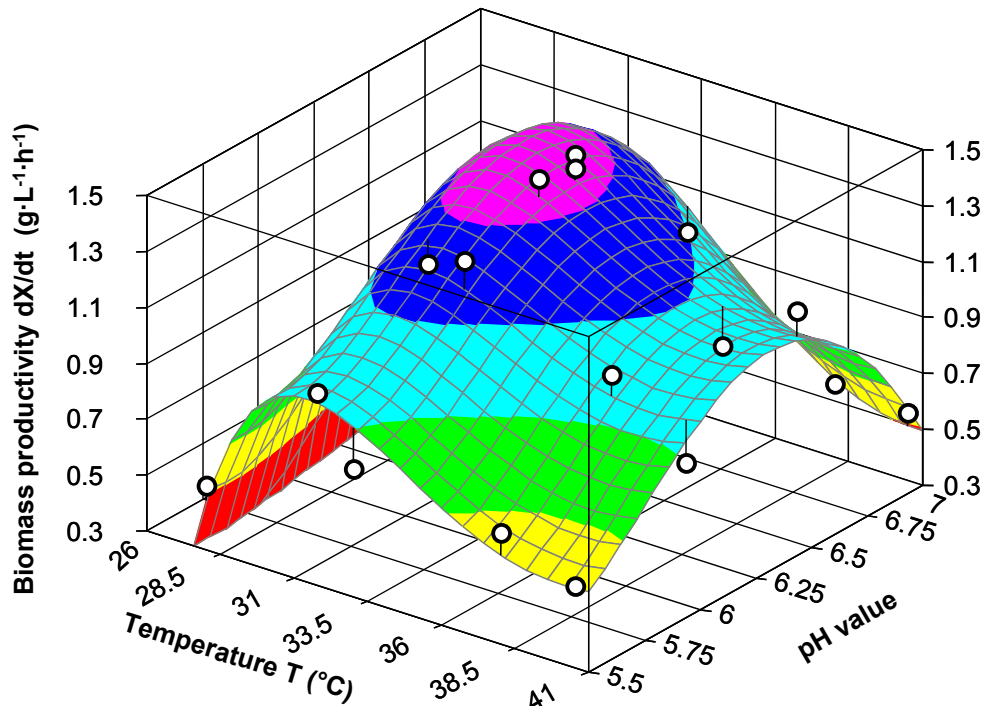


Figure 17: Spherical diagram of the function $dX/dt = f(T, pH)$

2.4.2.4 Estimate of the maximum values of the specific performance rates of the strain *L. paracasei* 168 by means of non-linear regression

This part of our research served to estimate the true limits of the strain *L. paracasei* 168 relating to cell growth, product formation, and substrate utilization. Assuming the cultivation medium used did not contain any limiting or inhibiting factor, the maximum values of cell metabolism could be realized by the strain at the optimum of the process parameters. Therefore, the maximum values of the specific rates listed in **Table 6** were also used in parameter optimisation.

Looking at **Table 6**, it is noticeable that the maximum values of specific growth rate found in the experiments vary between 0.13 h^{-1} and 0.75 h^{-1} . It appears that the latter value is not the true maximum value. Indeed, non-linear regression results in a maximum value of 0.66 h^{-1} (**Figure 18**). This value corresponds well with the maximum growth rate of other lactic acid bacteria strains very good.

The optimisation equation is of the same type as used in parameter optimisation for process performance characteristics (equation 11):

$$\mu_{max} = a + \frac{b}{T} + \frac{c}{pH} + \frac{d}{T^2} + \frac{e}{pH^2} + \frac{f}{T \cdot pH} + \frac{g}{T^3} + \frac{h}{pH^3} + \frac{i}{T \cdot pH^2} + \frac{j}{T^2 \cdot pH} \quad (11)$$

The constants of this equation were calculated to be

$a = 80.914733$, $b = -2249.4008$, $c = -1130.8056$, $d = 88299.291$, $e = 663.9054$,
 $f = -4429.6555$, $g = -1023649.8$, $h = -16512.746$, $i = 5968.3341$, $j = 29360.395$,
 $(r^2 = 0.9641)$

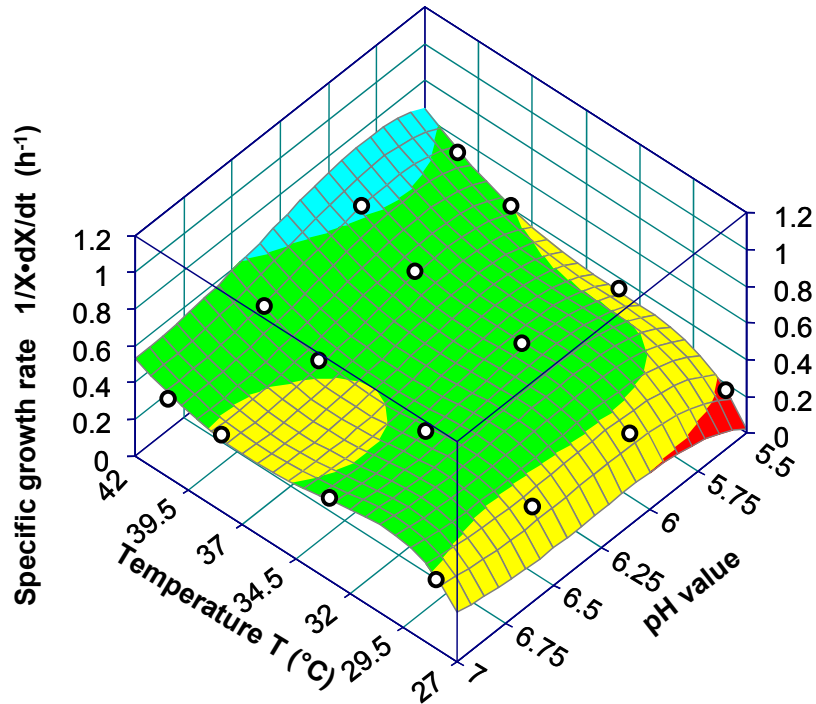


Figure 18: Spherical diagram of the function $\mu_{\max} = f(T, \text{pH})$

The maximum values of the specific lactic acid production rate found in these experiments lie between 1.17 and 3.0 h^{-1} . The result of an optimising calculation with these data is shown in **Figure 19**. The maximum specific lactic acid production rate is approximately 3.5 h^{-1} . This value corresponds to the maxima found with similar strains. The optimisation equation is identical with that used for optimising the specific growth rate, but its constants have other values:

$$\pi_{\max} = a + \frac{b}{T} + \frac{c}{\text{pH}} + \frac{d}{T^2} + \frac{e}{\text{pH}^2} + \frac{f}{T \cdot \text{pH}} + \frac{g}{T^3} + \frac{h}{\text{pH}^3} + \frac{i}{T \cdot \text{pH}^2} + \frac{j}{T^2 \cdot \text{pH}} \quad (12)$$

$a = 61.58855$, $b = -5599.4194$, $c = 297.37597$, $d = 31542.693$, $e = -8211.9485$,
 $f = 51352.903$, $g = 899659.61$, $h = 21766.745$, $i = -30139.076$, $j = -656507.72$,
 $(r^2 = 0.9236)$.

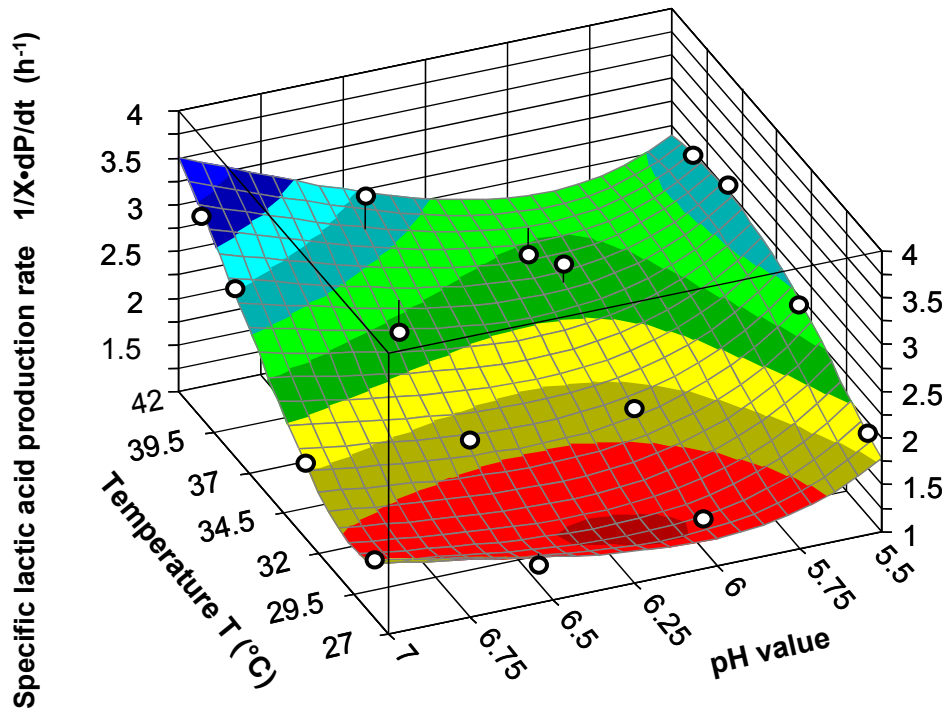


Figure 19: Spherical diagram of the function $\pi_{\max} = f(T, \text{pH})$

The maximum value of specific glucose utilization rate obtained in this particular experimental attempt varies between 1.15 and 4.46 h^{-1} (**Table 6**). Using equation (13) as a target function, non-linear regression with these values led to the following results (**Figure 20**):

$$\sigma_{\max} = a + \frac{b}{T} + \frac{c}{\text{pH}} + \frac{d}{T^2} + \frac{e}{\text{pH}^2} + \frac{f}{T \cdot \text{pH}} + \frac{g}{T^3} + \frac{h}{\text{pH}^3} + \frac{i}{T \cdot \text{pH}^2} + \frac{j}{T^2 \cdot \text{pH}} \quad (13)$$

$a = -87.863543$, $b = -9829.364$, $c = 3778.8257$, $d = 327070.51$, $e = -23816.727$,
 $f = -6244.8761$, $g = -3398841.5$, $h = 50525.632$, $i = 15912.146$, $j = -14421.084$,
 $(r^2 = 0.9776)$.

There is a top value for specific glucose utilization rate (3.72 h^{-1}) at a temperature of 41°C and neutral pH, which corresponds well with the maximum value of specific lactic acid production rate (**Figure 19**). In contrast to this, a real maximum value of specific glucose utilization rate exists at pH 5.5 and $T = 41^\circ\text{C}$. In this range glucose consumption and lactic acid production are not in correspondence.

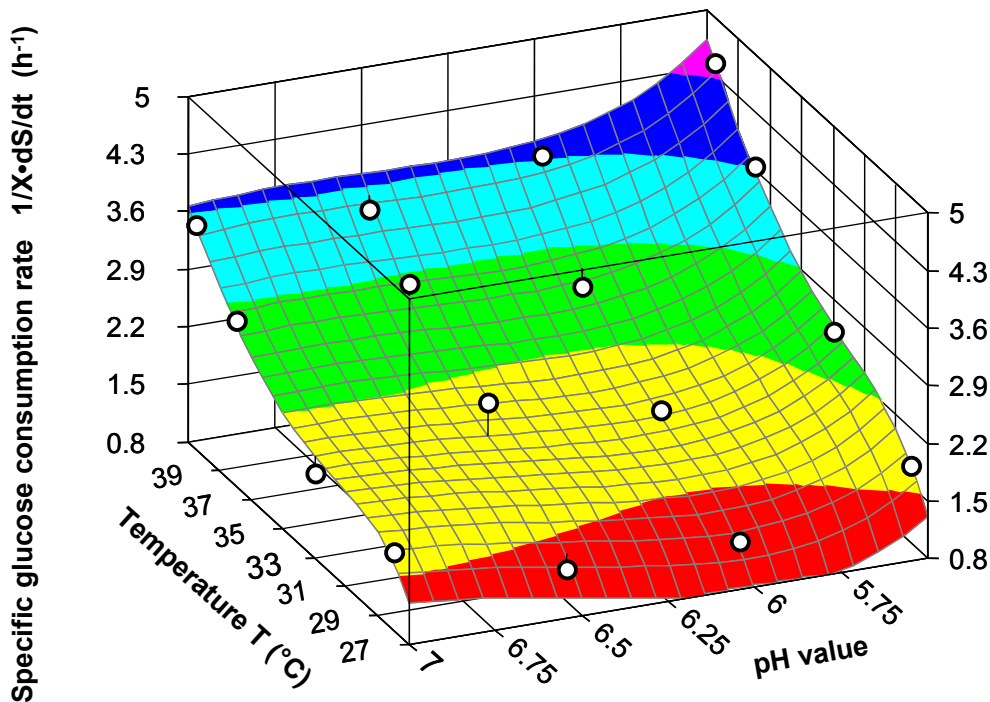
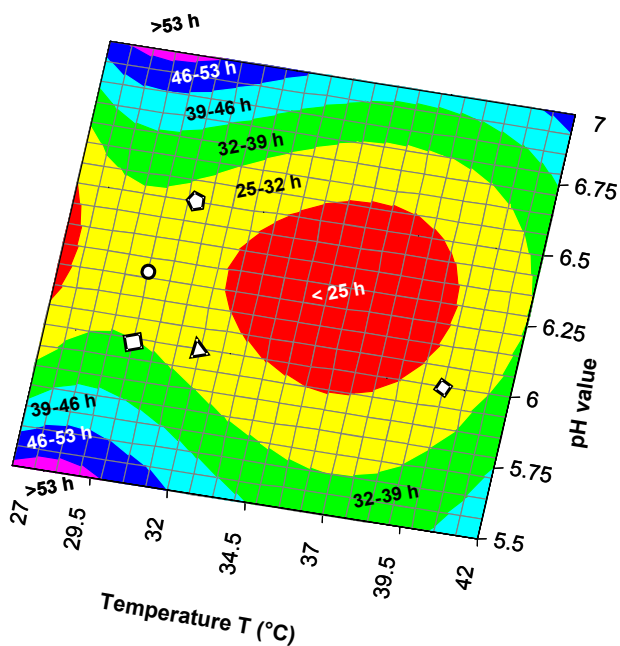


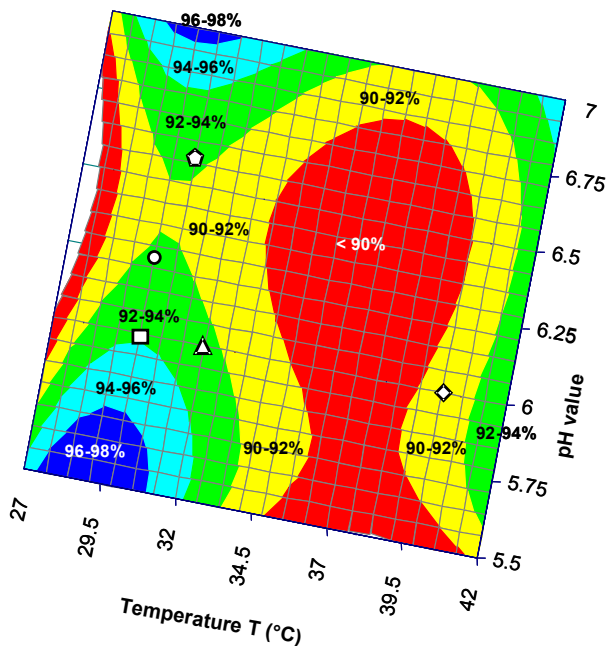
Figure 20: Spherical diagram of the function $\sigma_{\max} = f(T, \text{pH})$

2.4.3 Discussion of results and conclusions

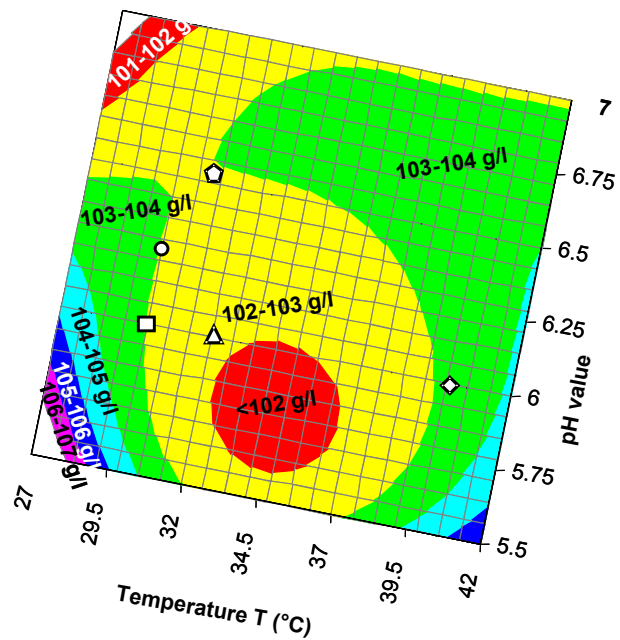
The desired result should be a process characterized by a minimum of fermentation duration, a maximum final lactic acid concentration, a maximum lactic acid yield, and maximal lactic acid productivity. It does not appear possible to discover a process parameter combination (T, pH) that guarantees the best values for all of the performance parameters. The situation is clearly illustrated in **Figure 21**. This figure shows the top view optimisation diagrams of the four process performance characteristics mentioned above. The minimum of process duration and the maximum of lactic acid productivity lie close together, but the lactic acid yield shows a significant minimum within this parameter range. Maximum lactic acid productivity and minimum duration of fermentation can be approximately realized, but this would result in a lactic acid yield of less than 90%. High lactic acid yields can be attained at lower temperatures under acidic or neutral conditions. A maximum of lactic acid yield is situated at a temperature and pH value of approximately 29°C and 5.5 respectively. This corresponds with the parameter range in which, high final lactic acid concentrations of more than 105 g · L⁻¹ are formed. The disadvantage is the long process duration (> 50 h). In the neutral range, high lactic acid yields are also coupled with high process durations and additionally with moderate final lactic acid concentrations.



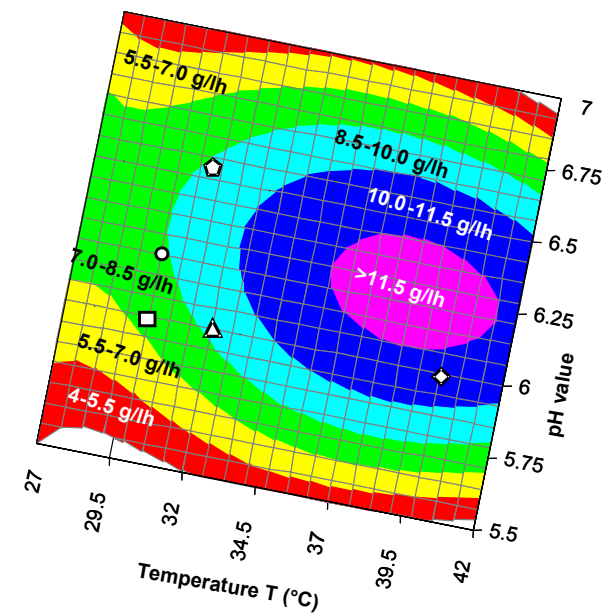
Duration of fermentation Δt (h)



Lactic acid yield Y_p (%)



Final lactic acid concentration P_{max} (g/l)



Lactic acid productivity dP/dt ($g \cdot L^{-1} \cdot h^{-1}$)

Fig. 21: Top view optimisation diagrams of the process performance characteristics when the strain *L. paracasei* 168 is cultivated on MRS medium

Within the entire parameter field investigated, the final lactic acid concentration never decreases below $100 \text{ g} \cdot \text{L}^{-1}$. Final lactic acid concentrations of 102 to $104 \text{ g} \cdot \text{L}^{-1}$ can be realized over a wide parameter range. To obtain lactic acid yields of 90 to 92% different parameter combinations can be used. Moreover, the duration of fermentation reaches to 25 to 39 h due to the decreasing lactic acid productivity. The exact results of optimisation are summarized in **Table 7**.

Table 7: Results of parameter optimisation with the strain *L. paracasei 168*

Performance parameter	Maximum of performance parameters			Minimum of performance parameters		
	Optimum T (°C)	Optimum pH	Value	Optimum T (°C)	Optimum pH	Value
Lactic acid productivity ($\text{g} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$)	39	6.25	11.8	There is no optimum		
Lactic acid Yield (%)	28.5	5.58	97.1	35	6.45	87.1
Final lactic acid concentration ($\text{g} \cdot \text{L}^{-1}$)	There is no optimum			35	5.8	101.6
Duration of Fermentation (h)	There is no optimum			37	6.2	21.8
Biomass productivity ($\text{g} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$)	31.4	6.52	1.41	There is no optimum		

Table 8 shows some possible working points of fermentation (also see the marked points in **Figure 21**). The selection of these points occurred under the premises that lactic acid yield, process duration, final lactic acid concentration, and maximum lactic acid productivity must be

- $Y_P \geq 92 \%$,
- $\Delta t \leq 32 \text{ h}$,
- $P_{\text{final}} \geq 102 \text{ g} \cdot \text{L}^{-1}$, and
- $dP/dt \geq 7 \text{ g} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$, respectively.

Table 8: Some possible parameter combinations for cultivating the strain *L. paracasei* 168 in batch fermentation

Performance parameter	T 41°C pH 6.0	T 32°C pH 6.0	T 31°C pH 6.5	T 30°C pH 6.25	T 30°C pH 6.0
	Results predicted				
Lactic acid productivity (g · L ⁻¹ · h ⁻¹)	10.8	8.8	8.9	8.5	7.6
Lactic acid Yield (%)	92.0	92.5	92.0	92.4	93.8
Final lactic acid Concentration (g · L ⁻¹)	103	102	103	103	103
Duration of Fermentation (h)	29.5	28.6	29.8	28.5	32.0

Process performance is always determined by two factors: the amount of active cell mass in the cultivation medium and the actual specific performance behaviour of this active cell mass. The two factors depend on the process and environment parameters that act on the active cells. For instance, the productivity of lactic acid formation is the product of cell mass and specific lactic acid production rate. For the maximum productivity we can write theoretically:

$$\left[\frac{dP}{dt} \right]_{max} = \left(\frac{1}{X} \cdot \frac{dP}{dt} \right)_{max} \cdot X_{max} = \pi_{max} \cdot X_{max} \quad (14)$$

In equation (14), the maximum specific lactic acid production rate π_{max} is a strain-specific performance characteristic, which can be realized by the microorganisms only under optimal process conditions and when no limiting or inhibitory factor affects the biological system. In contrast to this, the maximum cell concentration is a related quantity depending on the actual conditions of growth and technological actions (cell immobilization etc.).

The top view optimisation diagrams of the specific growth rate, the specific lactic acid production rate, and the specific glucose utilization rate are demonstrated in **Figure 22**.

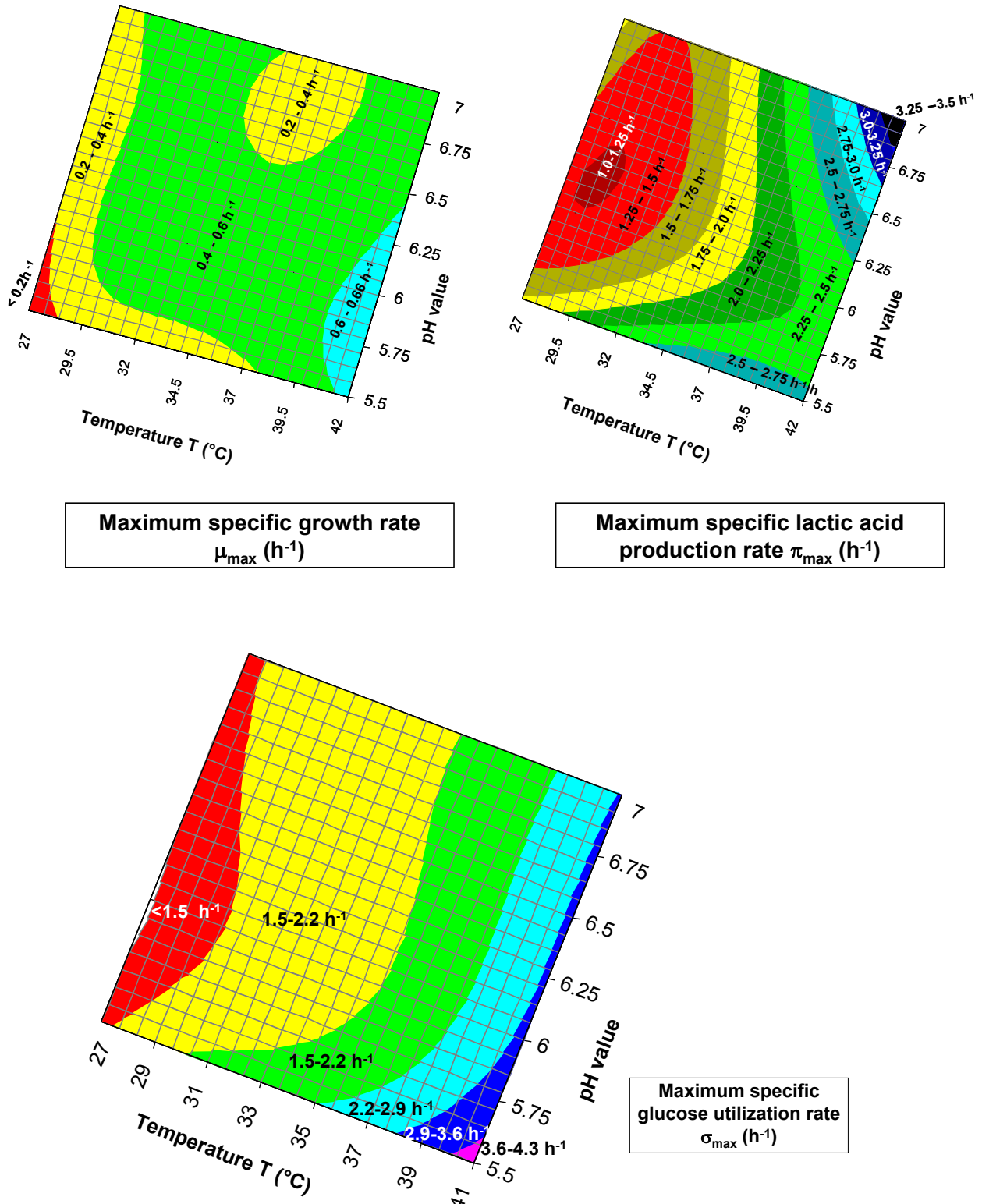


Figure 22: Top view optimisation diagrams of the specific performance characteristics of the strain *L. paracasei* 168 in batch cultivation

Comparing the three diagrams of the **Figure 22**, the following facts are noticeable:

- there is the same tendency in increasing the maximum possible values of specific growth rate, specific lactic acid production rate, and specific glucose utilization rate at increasing temperature, which means that cell growth and lactate formation are metabolically coupled and that the glucose utilized is mainly converted into biomass and lactic acid,
- there is a correspondence between the position of the maxima of specific growth rate and specific glucose utilization rate at higher temperatures in a weakly acid medium, which means that cell growth is optimal under these conditions,
- there is a significant difference between the positions of the maxima of specific lactic acid production rate (neutral range) and the specific glucose utilization rate (weakly acid range), which means that lactic acid formation is optimal in a neutral medium and that possibly the ratio of cell growth and lactic acid formation is altered in favour of biomass production under weakly acid conditions.

Considering all of the results described in this report, we can define the true performance limits of the strain *L. paracasei* 168:

- Maximum specific growth rate $\mu_{\max} = 0.66 \text{ h}^{-1}$
- Maximum specific lactic acid production rate $\pi_{\max} = 3.50 \text{ h}^{-1}$
- Maximum specific glucose utilization rate $\sigma_{\max 1} = 4.30 \text{ h}^{-1}$
- Maximum specific glucose utilization rate at π_{\max} $\sigma_{\max 2} = 3.72 \text{ h}^{-1}$

In conclusion it can be said that the strain *Lactobacillus paracasei* 168 possesses a high production capacity, which makes this strain suitable for using as a production strain. Further investigations should focus on testing its performance behaviour under technical conditions.

The strain *Lactobacillus paracasei* 168 was purchased from the German Collection of Microorganisms and Cell Cultures Braunschweig. The strain was isolated from silages by Chemische Werke Hül's AG and can be acquired by everybody. Patenting is not possible.

3 Preparation of glucose from barley grains

Starch hydrolyzates from cereals are very good raw materials for fermentation processes because they contain about 60 % of starch and can be made available through the entire year in best quality. It is known that barley may be an excellent raw material for lactic acid fermentation, but it is not used in large-scale lactate production till now. In the European Community, a quantity of about 48 million tons of barley corresponding to a quarter of the total grain production was harvested in 2003. The average yield in the period from the year 2000 to 2003 amounted up to 4,57 tons per hectare for the EU-15 countries [EU, 2005].

Cereals are very good raw materials for fermentation processes. They contain about 60 % of starch and can be made available through the entire year in best quality. The mainly used cereals are maize, rye and wheat in this context. In Iceland, the crop production is still low. About 3.000 t of cereal grains (mainly barley) were produced in 2000 only, but there are strategies for expanding this production next years. In Ireland, cereal production is a traditional part of agriculture. About 1.277.000 t of barley were produced in 2001/2002. Therefore, lactic acid production should be based on barley in a biorefinery that will be realized in these two countries. For a possible new facility that will be situated in Germany, rye will be the favourable raw material. In this report, the possibilities of the usage of barley as a raw material for lactic acid fermentation are discussed.

Cereal grains' starch is covered with hard, dry layers composed of cellulose. To use this starch, the outer structure must be broken up at first. General starch can't be used by lactic acid bacteria directly. For this purpose, the big starchy macromolecules have to be splitted down into glucose molecules by hydrolysis.

3.1 Grain milling

Winter barley purchased at an agricultural trade company in Brandenburg was milled with a roller mill (Neostat Neuhaus, Germany) in portions of 15 kg. The coarse meal obtained was analyzed in respect of its particle size spectrum. The results of sieve analysis carried out with a sieve shaker (KS 1000, Retsch, Germany) are presented in **Table 9**.

Table 9: Results of sieve analysis

Screen size [mm]	> 2 mm	> 1.4 mm	> 1 mm	> 0.5 mm	< 0.5 mm
Mass [g]	21	48	138	500	293

The first two fractions (screen size > 1.4 mm) containing mainly husks and other cellulosic material were separated and discarded. The remaining material (93.9 % of total mass) was used for enzymatic hydrolysis. A substance balance of this procedure is shown in **Figure 23**.

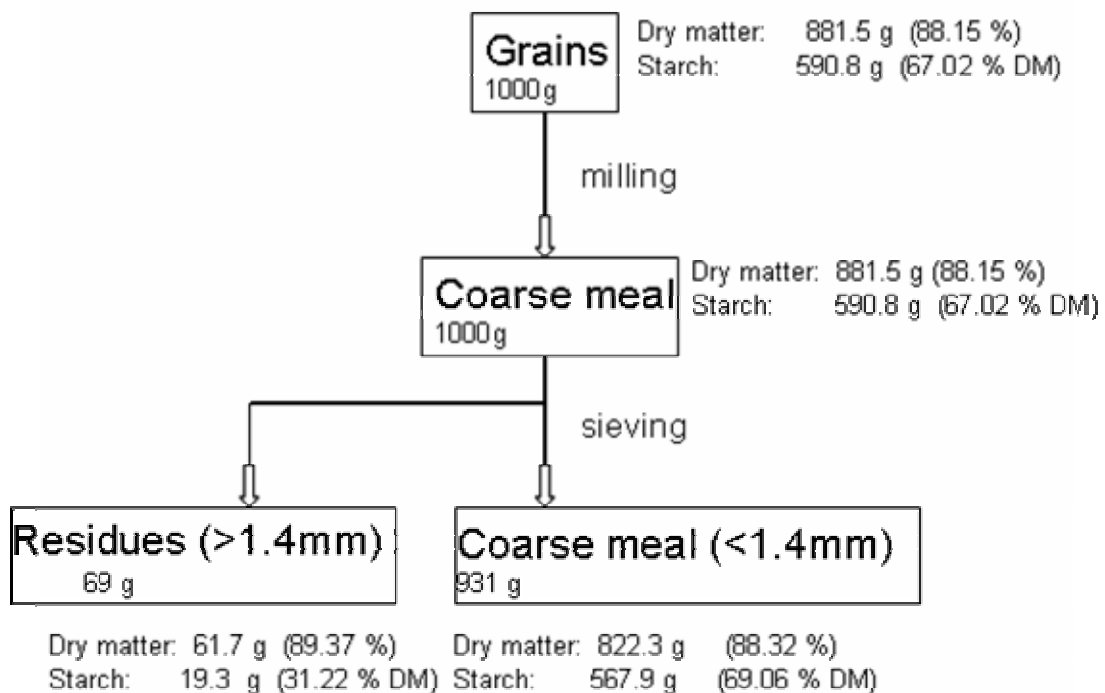


Figure 23: Substance balance of grain milling and sieving

96 % of the original starch contained in the grains were made available for enzymatic hydrolysis.

3.2 Enzymatic hydrolysis of barley coarse meal

The hydrolysis occurs in two steps. First, the insoluble starch must be liquefied at higher temperatures (80 - 110°C) by means of an α -amylase and secondly, the liquified starch is then degraded down to glucose by a glucoamylase at medium temperatures (40 - 60°C). The resulting hydrolysate contains > 130 g of glucose per litre and some solid residues of non-starchy matter. This mixture can be used in this form as medium in batch fermentation. For use in high-performance continuous fermentation, a separation of solids by filtration is recommended.

In this research, the process parameters (temperature, pH value and process duration) of the two hydrolysis stages were optimized using experimental designs.

3.2.1 Starch liquefying

For starch liquefying the commercial enzyme preparation Termamyl 120L from Novo Nordisk (DK) was used. It is an α -amylase which splittet starch into oligosaccharides consisting of 5 - 7 glucose units. The enzyme is from the bacteria *Bacillus licheniformis* and is offered as a dark-brown aqueous solution.

The experiments were carried out in a 2-litre-laboratory stirred vessel reactor equipped with devices for controlling both the temperature and the pH value.

250 g of the barley coarse meal (particle size < 1,4 mm) was suspended under stirring (500 rpm) into 1 litre of warm water (45°C). Then the mixture was heated up to the foreseen temperature and pH was adjusted at its desired value. Immediately after that, 10 mL of the enzyme was added and the resulting mixture was stirred under the conditions mentioned for 4 hours. The initially high-viscous solution becomes highly liquid during this time. Straight after ending the trial, the mixture was prepared for chemical analysis. After liquid/solid separation in the solid and the liquid the contents of starch, dry matter, raw protein and glucose, maltose respectively, were determined.

In previous investigations carried out in the ATB, liquefying of rye and wheat coarse meal was optimized. In this research it was found that adding 10 mL of the enzyme to the mixture and a process duration of 4 hours resulted in a high degree of starch liquefaction (98 – 99 %). Therefore, these two parameters were so also accepted as constants in the optimization of barley starch liquefying. The parameters varied were temperature and pH value. The ranges of variation were $70^{\circ}\text{C} \leq T \leq 90^{\circ}\text{C}$ and $5.0 \leq \text{pH} \leq 7.0$. The experimental design (**Figure 24**) contained 16 trials with the following parameter combinations:

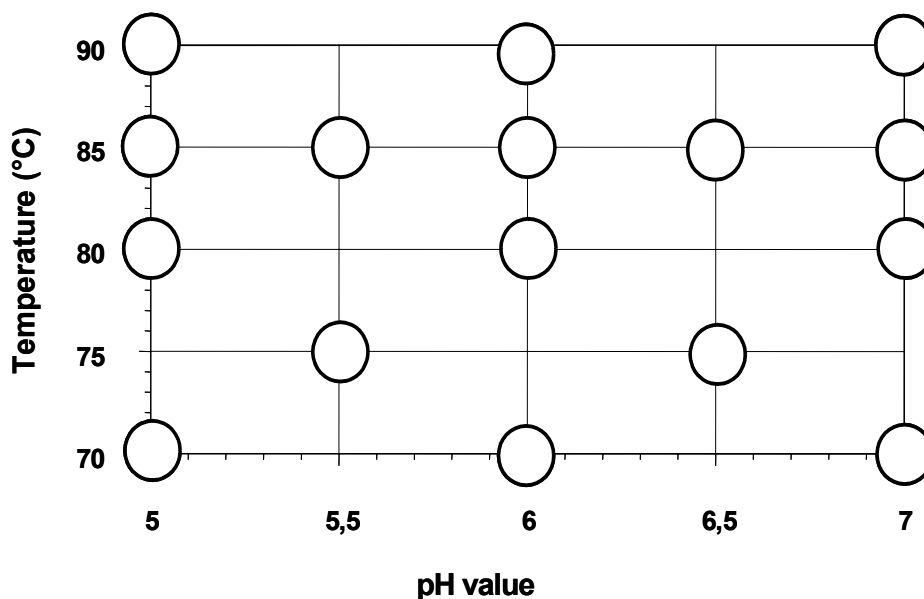


Figure 24: Experimental design in starch liquefying

The results obtained in the experiments are listed in **Table 10**. In contrast to rye starch hydrolysis, the degree of starch liquefaction was lower. The minimum content of starch in the solid residues amounted to 6.9 %. This corresponds with a degree of starch liquefaction of 95.3 %.

Table 10: Results obtained in barley starch liquefaction

Temperature	pH value	Content of dry matter in solids	Residual starch content of solids	Content of raw protein in solids	Content of glucose in liquid	Content of maltose in liquid	Degree of starch liquefaction
[°C]	[-]	[%]	[% DM]	[% DM]	[g · L ⁻¹]	[g · L ⁻¹]	[%]
70	5.0	19.54	12.6	16.43	7.30	32.31	85.0
70	6.0	19.61	23.7	16.56	6.92	31.99	88.9
70	7.0	20.91	20.9	16.5	6.46	25.88	87.75
75	5.5	25.68	14.9	12.87	8.79	31.86	90.21
75	6.5	23.91	16.0	13.25	8.40	31.61	89.59
80	5.0	21.05	20.8	13.0	8.68	30.68	86.52
80	6.0	22.15	14.8	14.0	9.31	31.20	90.25
80	7.0	21.50	12.7	14.0	9.32	34.03	91.77
85	5.0	20.37	10.7	13.31	5.65	31.93	92.53
85	5.5	21.44	12.8	14.5	4.92	24.24	91.88
85	6.0	23.93	8.0	11.19	6.10	22.48	94.92
85	6.5	23.85	6.9	12.94	7.07	25.90	93.15
85	7.0	21.05	7.8	14.25	8.52	35.47	92.53
90	5.0	20.66	9.64	-	10.28	36.63	94.12
90	6.0	23.17	7.57	-	13.43	37.71	95.29
90	7.0	23.16	8.03	-	14.67	33.63	94.5

A higher degree of starch liquefaction (98 %) is predicted for higher temperatures (T = 100°C) by the model (**Figures 25, 26**), obtained from the measured data by means of a regression computer program.

$$DSL = a + \frac{b}{T} + \frac{c}{(\text{pH})} + \frac{d}{T^2} + \frac{e}{(\text{pH})^2} + \frac{f}{T \cdot (\text{pH})} + \frac{g}{T^3} + \frac{h}{(\text{pH})^3} + \frac{i}{T \cdot (\text{pH})^2} + \frac{j}{T^2 \cdot (\text{pH})}$$

$$a = -457.29908; b = 186633.92; c = -3863.5552; d = -14375070; e = 30732.523; \\ f = -145914.98; g = 3.1916698e+08; h = -44390.83; i = -813390.34; j = 16157390.$$

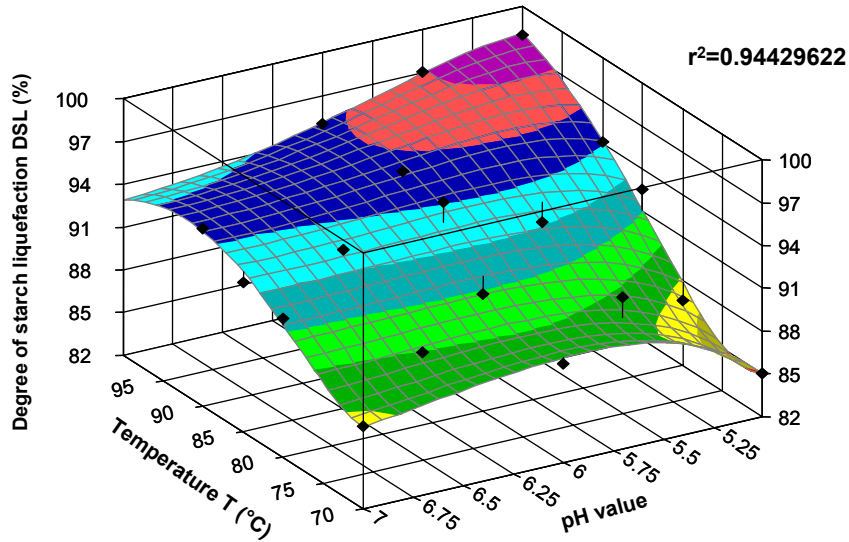


Figure 25: Dendence of the degree of starch liquefaction in barley coarse meal hydrolysis with Termamyl 120 L on temperature and pH value

$$S_{\text{solid}} = a + \frac{b}{T} + \frac{c}{(\text{pH})} + \frac{d}{T^2} + \frac{e}{(\text{pH})^2} + \frac{f}{T \cdot (\text{pH})} + \frac{g}{T^3} + \frac{h}{(\text{pH})^3} + \frac{i}{T \cdot (\text{pH})^2} + \frac{j}{T^2 \cdot (\text{pH})}$$

$$a = 3391.2406; b = -559073.5; c = 19849.17; d = 27528237; e = 20549.124 \\ f = 2639149.7; g = -4.3409095e+08; h = 29848.552; i = -2854073; j = -67237408$$

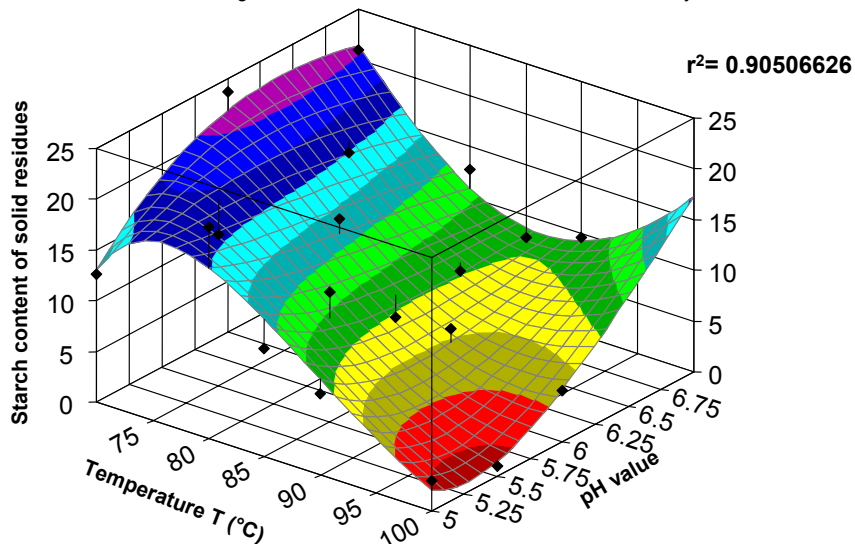


Figure 26: Dependence of the starch content in the residual solid after starch liquefaction of barley coarse meal with Termamyl 120 L on temperature and pH value

3.2.2 Starch saccharification

For starch saccharifying the commercial enzyme preparation AMG 300 L from Novo Nordisk (DK) was used. It is an glucoamylase which splittet oligosaccharides into glucose. The enzyme is from the fungus *Aspergillus niger* and is offered as a clear brown aqueous solution.

Experiments for starch saccharification were carried out in the same 2-litre-stirred vessel reactor which was used in starch liquefying previously. In all of the trials, the same starting material was used. The latter was produced in a single liquefying process using a 50-litre-stirred tank reactor. The liquefied starch obtained was stored in small portions of 1 litre in a freezing room at -21°C .

Immediately before use 1 litre of the frozen starch solution was thawed and then heated up to the temperature desired under stirring (500 rpm). After adjusting the pH value at its set point, 10 mL of the commercial enzyme preparation AMG 300 L was added and the mixture was incubated for 4 hours under these conditions with sampling after each hour. The samples were immediately deactivated by heating in a boiling water bath and then analyzed in respect of its glucose content. The parameters varied were temperature and pH value.

The ranges of parameter variation were $40^{\circ}\text{C} \leq T \leq 60^{\circ}\text{C}$ and $3.0 \leq \text{pH} \leq 5.0$. The experimental design (**Figure 27**) contained 13 trials with the following parameter combinations:

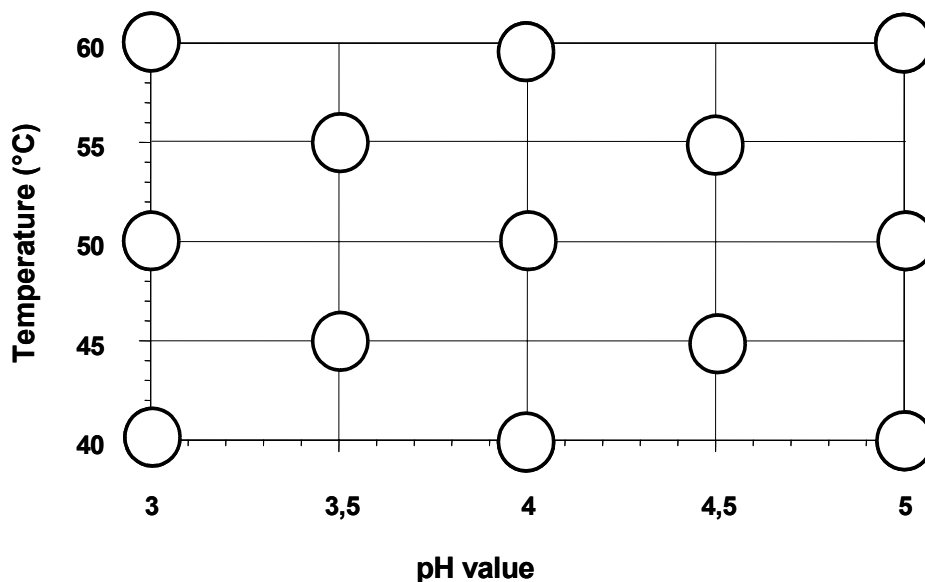


Figure 27: Experimental design in starch saccharification

The results obtained in the experiments are listed in **Table 11**. The minimum content of starch residue in the samples at different conditions amounted to 3.45 g. This corresponds with a degree of starch residue saccharification of about 80 %.

Table 11: Results obtained in barley starch saccharification

Temperature	pH value	Starch residue	Degree of starch residue saccharification	Glucose after 1 h	Glucose after 2 h	Glucose after 3 h	Glucose after 4 h
[°C]	[-]	[g]	[%]	[g · L ⁻¹]	[g · L ⁻¹]	[g · L ⁻¹]	[g · L ⁻¹]
60	5.0	9.56	44.07	124.2	132.9	120.4	126.1
60	4.0	5.95	65.22	129.0	133.0	133.7	128.6
60	3.0	7.05	58.78	126.7	128.5	128.9	132.3
40	5.0	7.25	57.61	129.6	134.3	133.3	130.5
55	4.5	6.49	62.07	129.7	126.8	126.5	126.3
55	3.5	6.41	62.52	126.8	128.0	125.1	122.7
50	5.0	6.94	59.44	126.9	127.9	128.1	127.4
50	4.0	9.65	43.56	126.9	130.6	127.5	130.2
50	3.0	6.57	61.59	124.9	119.6	124.6	131.2
40	3.0	3.45	79.81	122.7	121.8	123.2	120.6
40	4.0	5.74	66.40	121.4	121.5	114.4	120.2
45	3.5	8.17	52.24	133.2	124.0	126.4	123.2
45	4.5	6.49	62.03	122.9	125.6	124.5	124.2

The degree of starch residue saccharification (DSS) can be described with a model of the same structure like the process of liquefaction. Using the corresponding values from **Table 11** the model approximation ($r^2 = 0.654$) results in a model equation which is illustrated in **Figure 28**:

$$DSS = a + \frac{b}{T} + \frac{c}{pH} + \frac{d}{T^2} + \frac{e}{pH^2} + \frac{f}{T \cdot pH} + \frac{g}{T^3} + \frac{h}{pH^3} + \frac{i}{T \cdot pH^2} + \frac{j}{T^2 \cdot pH} \quad (15)$$

with the coefficients:

$$a = -2.444,58; b = 202.435,5; c = 3.528,33; d = -7.141 \cdot 10^6; e = -33.098,1; \\ f = -474.210,28; g = -9.327 \cdot 10^7; h = 30.954,19; i = 411.516,9; j = 6.246 \cdot 10^6.$$

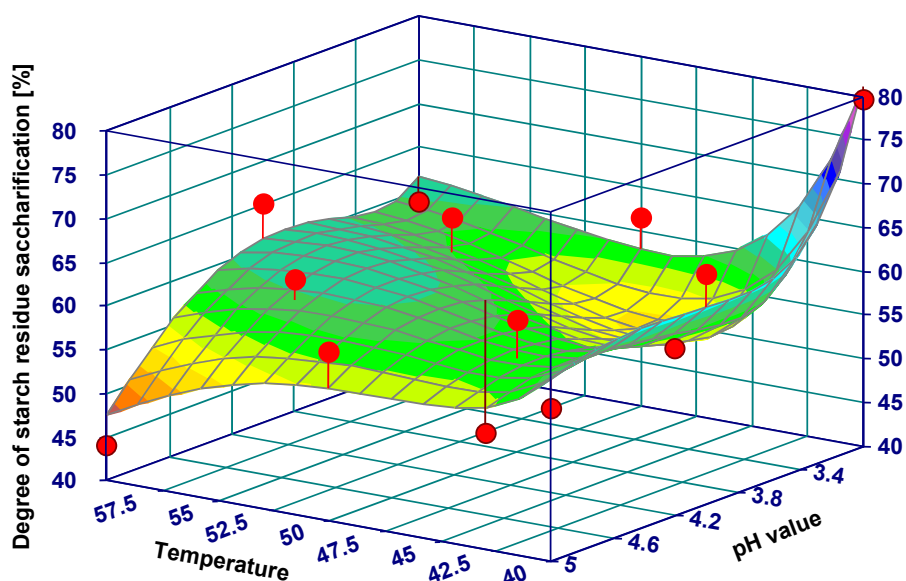


Figure 28: Dependency of the degree of starch residue saccharification (DSS) on temperature and pH value

In view of preparing a basic nutrient (carbon source) for the fermentation of lactic acid bacteria to produce lactic acid one of the most important parameters was the accessible concentration of glucose at the end of the hydrolysis. It can be seen from the values of **Table 11** that the highest values of glucose concentration were between 130 and $135 \text{ g} \cdot \text{L}^{-1}$ respectively.

Diagrams of the previous model for dependency on the parameters temperature and pH value at different times are illustrated in **Figure 29** and **30**.

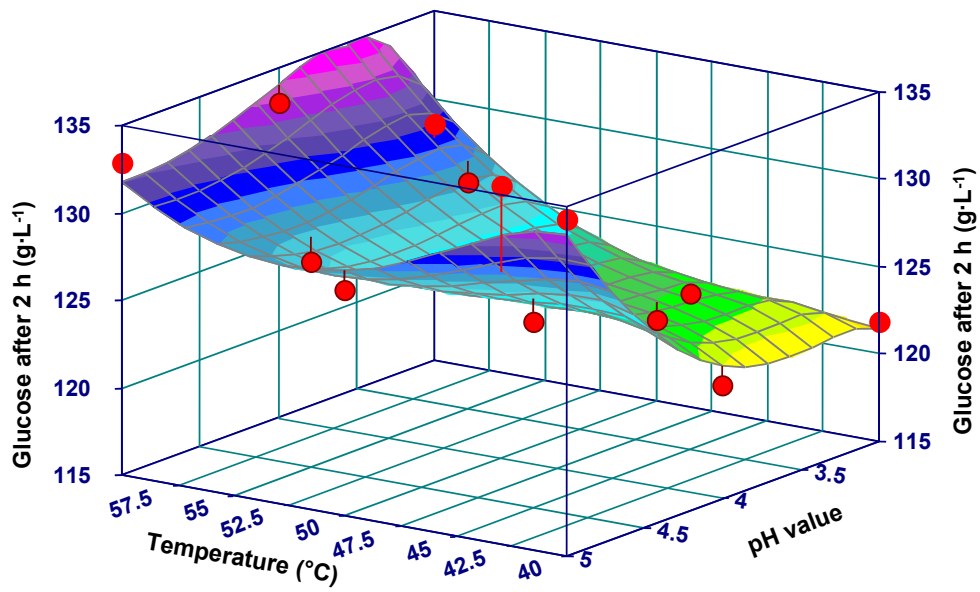


Figure 29: Glucose concentration after 2 hours of saccharification process

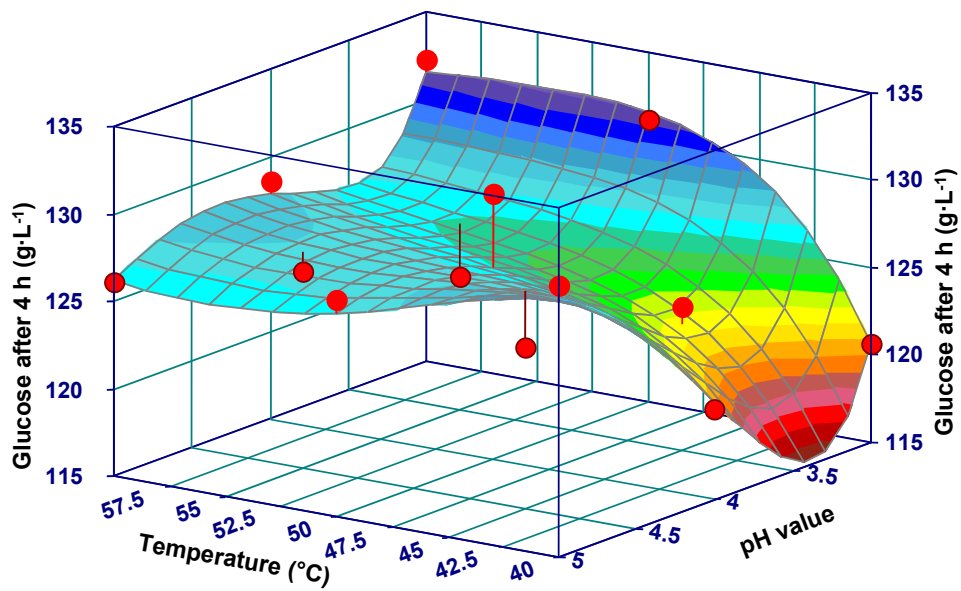


Figure 30: Glucose concentration after 4 hours of saccharification process

To optimize the highest possible glucose concentration it was necessary to consider not only the process parameters temperature and pH value but also the duration of the saccharification. With the above mentioned 3-dimensional model only three parameters can be correlated in each trial. Therefore the raw data were divided in three groups which one of these parameters as constant. A summary of the results of modeling is given in **Table 12**.

Table 12: Results of optimization of the conditions for the maximum glucose concentration after saccharification

Temperature [°C]	pH value [-]	Time [hours]	max. glucose concentration [g · L ⁻¹]	Condition of modeling
60.0	3.65	1	131.2	time = const.
60.0	3.75	2	134.9	
60.0	3.71	3	134.1	
42.6	5.0	3	135.7	
54.3	3.0	4	131.6	
40.0	5.0	4	129.7	
40.0	5.0	1.36	137.2	Temperature = const.
50.0	5.0	1.30	134.1	
50.0	3.42	4	132.2	
60.0	3.95	1.30	142.3	
60.0	3.0	4	133.1	pH value = const.
60.0	4.0	1.30	138.1	
40.0	5.0	1.23	141.8	

The optimum of the time t for saccharification is about 1,3 hours. The highest possible glucose concentration ($c_{\max.}$) at these conditions comes to $142.3 \text{ g} \cdot \text{L}^{-1}$ if the temperature is high (60°C) and the pH value is moderate (about 4,0). Nearly the same result is reachable at lower temperature (40°C) and higher pH-value (5,0)

1. pH = 3.95; T = 60°C ; t = 1.3 hours; $c_{\max.} = 142.3 \text{ g} \cdot \text{L}^{-1}$
2. pH = 5.00; T = 40°C ; t = 1.3 hours; $c_{\max.} = 141.8 \text{ g} \cdot \text{L}^{-1}$.

An other maximum within the evaluated parameter range exists at low pH value (3,0) and high temperature (60°C). But for that a period of time of 4 hours for saccharification is necessary:

3. pH = 3.0 ; T = 60°C ; t = 4.0 hours; $c_{\max} = 133.1 \text{ g} \cdot \text{L}^{-1}$

In view of saving energy costs the best result of optimization is the second variation. The third one needs a longer time and does not lead to the yields of the others (compare also **Table 12**).

3.3 Mass balance of starch hydrolysis

From starch liquids with a potential content of $134 \text{ g} \cdot \text{L}^{-1}$ glucose solutions with a concentration of $133.7 \text{ g} \cdot \text{L}^{-1}$ were prepared in a realistic hydrolysis process. This corresponds to a degree of saccharification of about 90 %. Together with the results from the optimization it should be possible to prepare glucose solutions within a concentration range between 140 and $145 \text{ g} \cdot \text{L}^{-1}$ respectively. In that way the degree of saccharification can be increased up to 95 % (**Table 13**).

Table 13: Summary of the results

Starch content at the beginning	$134.0 \text{ g} \cdot \text{L}^{-1}$
Theoretical maximum of glucose concentration	$149.0 \text{ g} \cdot \text{L}^{-1}$
Maximum of glucose after optimization	$141.8 \text{ g} \cdot \text{L}^{-1}$
Practical maximum of glucose concentration	$133.7 \text{ g} \cdot \text{L}^{-1}$
Theoretical degree of saccharification	100 %
Maximum degree of saccharification after optimization	95.1 %
Practical degree of saccharification	89.7 %
Theoretical recoverable yield of glucose from 1 kg wholemeal	487.1 g
Maximum recoverable yield of glucose from 1 kg wholemeal	463.3 g
Practical recoverable yield of glucose from 1 kg wholemeal	436.9 g

4 Utilization of green biomass as nitrogen source

4.1 Preparation of green juice from wet lucerne by pressing

According to the intention to substitute expensive nutrient supplements by cheaper materials from renewable resources there were tested green material from lucerne for the cultivation of lactic acid bacteria. For the lactic acid fermentation with suspended cells in a stirred bioreactor it was necessary to prepare a liquid with a certain content of nitrogen and raw protein respectively. For that purpose fresh green material was pressed to separate the juice and the cake.

Lucerne was harvested by means of a chopper (fibre length of 10 cm) and immediately transported to the pressing place over a distance of 15 km. A screw press Cv (VETTER Maschinenfabrik GmbH & Co.KG, Kassel/Germany) with a capacity of 500 - 800 kg green mass per hour was used. In all, about 1 t of lucerne was pressed under a the half maximum press pressure resulting in more than 400 litres green juice. Pressing occurred without any difficulties (**Figure 31**). It may be assumed that a higher degree of dehydration is attainable when the press pressure will be increased. The juice was drawn off into 5-litre-plastic containers and stored in a refrigerating room at a temperature of -21°C .



Figure 31: Pressing of fresh green biomass (lucerne grass)

A substrat balance sheet of pressing is shown in **Figure 32**. In the pressing procedure carried out, 446 kg of green juice and 554 kg of a press cake were obtained from 1000 kg chopped lucerne. The juice and the solid residue had a dry matter content of 5,5 % (w/w) and 27.8 % (w/w), respectively. This means that more than a half of the total water containing in the green mass was separated as juice.

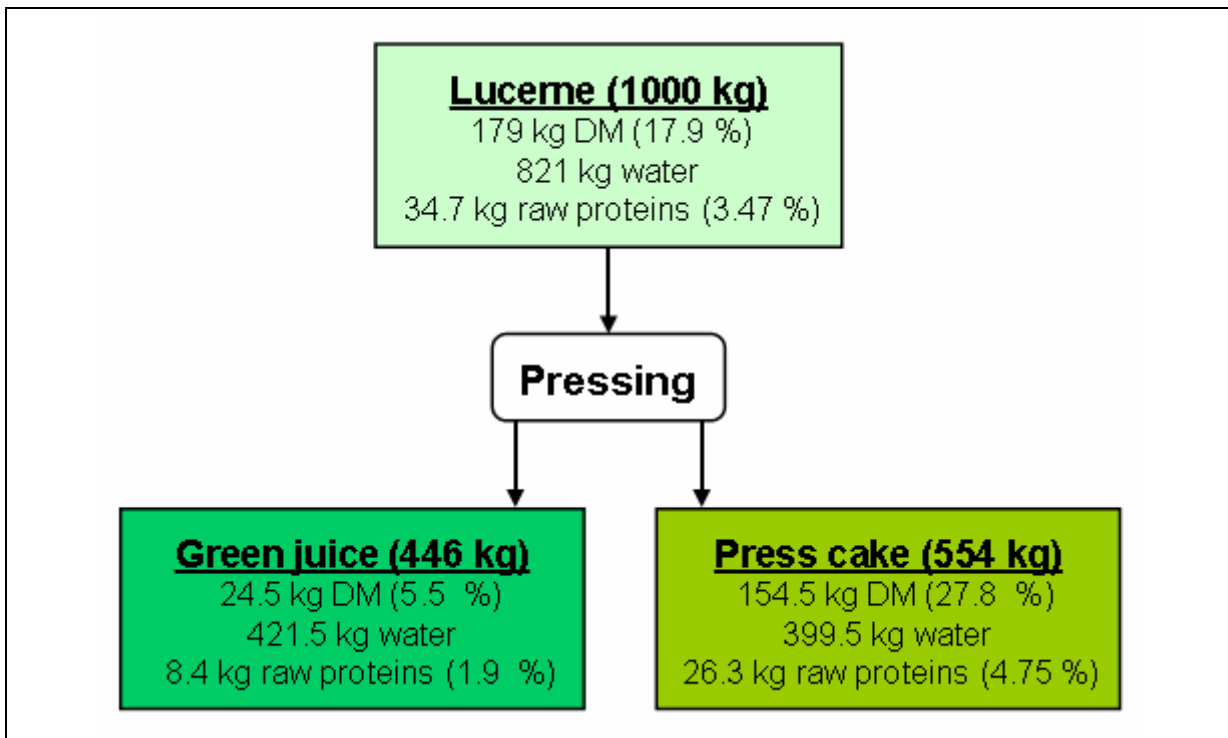


Figure 32: Substance balance of lucerne pressing

4.2 Chemical analysis of pressing products

An overview about the composition of the liquid and solid phases appearing in the pressing process is given in next tables. **Table 14** shows the total dry matter (DM) content and the content of organic mass in the dry matter (ODM) of the two solid phases and the juice. It is clear to see that the organic portion of dry matter increased in the solid and decreased in the juice compared to its value in the primary material. Besides, pH value, conductivity and chemical oxygen demand (COD) of them are presented.

Table 14: Results obtained in pressing freshly harvested lucerne green mass

	DM ₁₀₅ [%]	ODM [% DM]	pH	Conductivity [mS · cm ⁻¹]	COD [g · L ⁻¹ juice] [g · kg ⁻¹ cake]
Lucerne green mass	17.9	86.4	6.14	1.97	200
Lucerne green juice 1	5.37	76.0	6.05	17.60	69.0
Lucerne green juice 2	5.37	76.0	6.18	17.50	70.5
Lucerne green juice 3	5.37	76.0	6.14	17.50	69.8
Lucerne green juice 4	5.61	74.7	6.23	17.50	69.3
Lucerne green juice 5	5.61	74.7	6.18	17.70	68.9
Lucerne green juice 6	5.61	74.7	6.00	17.50	67.5
Lucerne green juice 7	5.64	75.4	6.17	17.80	68.2
Average	5.51	75.36	6.14	17.59	69.0
Lucerne press cake 1	29.0	78.4	6.03	1.98	347
Lucerne press cake 2	26.7	90.2	5.83	1.92	319
Lucerne press cake 3	26.4	91.1	5.93	2.00	355
Lucerne press cake 4	26.1	90.7	5.94	2.00	317
Lucerne press cake 5	26.2	89.9	6.02	1.94	333
Lucerne press cake 6	27.3	90.5	5.95	1.96	353
Lucerne press cake 7	32.9	91.6	6.07	1.95	402
Average	27.8	88.9	5.97	1.96	347

In **Table 15**, the data being necessary for a nitrogen balance are presented. The juice contains about 450 mg ammonium nitrogen per litre what corresponds with the nitrogen demand of about 4.5 g of bacterial dry mass per litre. The ammonium nitrogen is contained in the total nitrogen content. In order to calculate the content of raw protein, we have to multiply the difference of the two nitrogen values with the factor 6.25.

Table 15: Nitrogen contents of lucerne green juice and press cakes

	NH ₄ -N [g · kg ⁻¹]	NO ₃ -N [mg · L ⁻¹]	Total-N [g · kg ⁻¹]	Raw protein [g · kg ⁻¹]
Lucerne green mass	0.402	-	5,95	34.68
Lucerne green juice (7 samples)	0.436 - 0.503	< 0.25 - 0.88	3.43 - 3.51	18.68 - 19.04
Average	0.453	-	3.47	18.86
Lucerne press cake (7 samples)	0.293 - 0.458	-	5.91 - 9.92	34.43 - 59.41
Average	0.367	-	7.98	47.57

Table 16 shows the contents of carbohydrates in the lucerne and the two separated phases after pressing. The green juice cannot serve as carbon source for lactic acid production, because it contains a few sugars (total sugar content: $9.1 \text{ g} \cdot \text{L}^{-1}$) only. In a fermentation using green juice or its nitrogen containing components, the carbon source must be an external raw material like grains.

Table 16: Carbohydrate contents of lucerne green juice and press cakes

	Starch	Raw fibres	Sacch.	Glucose	Fructose
	[% DM]	[% DM]		[g · L ⁻¹ juice] [g · kg ⁻¹ cake]	
Lucerne green mass	4.6	34.5	0.172	0.210	0.061
Lucerne green juice (7 samples)	-	-	0.918 - 1.642	4.84 - 6.69	1.81 - 2.76
Average	-	-	1.161	5.69	2.32
Lucerne press cake (7 samples)	2.3 - 5.2	37.2 - 39.4	up to 0.287	0.34 - 0.42	up to 0.097
Average	2.97	38.67	0.282	0.379	0.0965

The green juice contains some salt components (phosphate, Mg^{++} , K^+ , Na^+) which are also needed by microorganisms for growth (**Table 17**). The total salt content amounts to about $10 \text{ g} \cdot \text{L}^{-1}$. Unfortunately there is also a portion of chloride of about $1.7 \text{ g} \cdot \text{L}^{-1}$ in the juice. This could lead to a problem in lactic acid purification when high-purity lactic acid should be produced. Besides the juice contains also about 2 g of organic acids (acetic acid, lactic acid) per litre.

Table 17: Contents of anorganic salts and organic acids in lucerne green juice and lucerne press cake

	Cl ⁻	SO ₄ ²⁻	PO ₄ ³⁻	Org. acids	Na ⁺	K ⁺	Mg ⁺⁺	Ca ⁺⁺
Lucerne green juice 1	1730	979	243	1400	85.4	5390	211	1400
Lucerne green juice 2	1740	864	229	1900	86.2	5410	213	1330
Lucerne green juice 3	1750	812	247	1800	83.4	5320	226	1430
Lucerne green juice 4	1750	828	242	2100	84.4	5450	230	1390
Lucerne green juice 5	1760	780	246	2300	90.6	5410	234	1430
Lucerne green juice 6	1720	877	240	2300	85.1	5410	234	1400
Lucerne green juice 7	1720	1020	230	2100	89.0	5880	240	1310
Average	1739	880	240	1943	86.3	5467	227	1384

5 Batch fermentation in laboratory and bench scale

After strain optimization at defined standard conditions it was to investigate the suitability of complex agricultural materials for the fermentation process. As noticed above, the green juice can't be used as carbon source in lactic acid fermentation. Therefore, barley hydrolysate is to use as raw material in this case. But the juice contains a series of nitrogen-containing compounds and anorganic salts, which are essential for cell growth. In this research, it was to test wheather the protein components of the juice can be used as nitrogen and nutrient source. The objective was to substitute the expensive yeast and meat extracts as well as peptone by the proteins containing in the green juice.

For testing of several substrate variations of grain hydrolyzate for cultivating the strain *L. paracasei* 168 more than 45 batch fermentation runs have been carried out:

- 13 with protein-enriched **lucerne juice**
- 9 with protein-enriched **lupine juice**
- 24 without green juice

There was no any impairment of growth and lactic acid production in these experiments. This means that barley hydrolysate can be used as the basic component (carbon source) for lactic acid fermentation. The macronutrients (nitrogen sources, mineral salts) were reduced and substituted by protein concentrate of the different green materials.

5.1 Raw materials for lactic acid fermentation

As noticed above, the green juice can't be used as carbon source in lactic acid fermentation. Therefore, barley hydrolysate is to use as raw material in this case.

But the juice contains a series of nitrogen-containing compounds and anorganic salts, which are essential for cell growth. In this research, it was to test wheather the protein components of the juice can be used as nitrogen and nutrient source. The objective was to substitute the expensive yeast and meat extracts as well as peptone by the proteins containing in the green juice. For solving this problem, we considered two possible technologies:

- using the complete juice as a blend of barley hydrolysate medium,
- separating and pretreating the proteins and adding thereof to the barley hydrolysate medium as a nutrient extract.

For the second variation the green juice (44.6 % of total lucerne biomass) was used for concentration of the raw protein by a nanofiltration step.

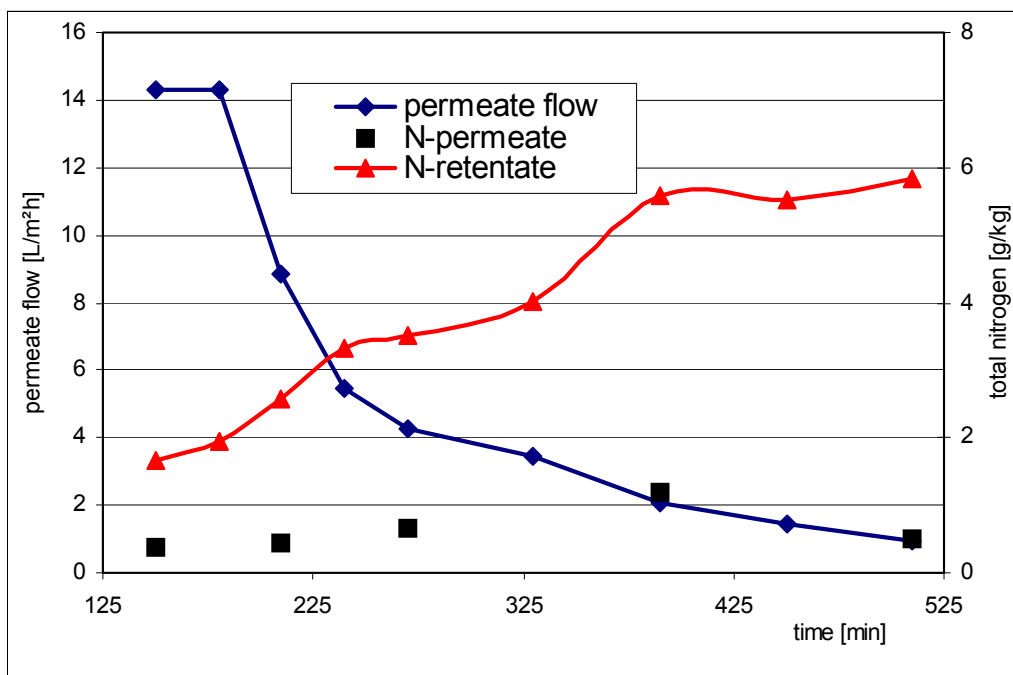


Figure 33: Nitrogen enrichment during the nanofiltration of green juice

It can be seen from **Figure 33** that nitrogen concentration (in this case in place of protein too) increased in retentate whereas the content in the permeate was still nearly the same. For further experiments it should be possible to select any other types of membranes to accelerate the concentration process and to get a higher degree of enrichment.

For the following fermentation trials the barley hydrolysate, the original green juice and the protein concentrate were used to prepare a basal nutrient medium for lactic acid bacteria.

5.2 Batch cultivation of a lactic acid bacterium on barley hydrolysate

Strain optimization of *Lactobacillus paracasei* 168 was carried out with a nutrient-enriched medium usable in microbiological laboratory practice. The composition of this MRS medium was yeast extract $5 \text{ g} \cdot \text{L}^{-1}$; peptone $10 \text{ g} \cdot \text{L}^{-1}$; meat extract $10 \text{ g} \cdot \text{L}^{-1}$; tween 80 $1 \text{ g} \cdot \text{L}^{-1}$; sodium acetate $5 \text{ g} \cdot \text{L}^{-1}$; ammonium citrate $2 \text{ g} \cdot \text{L}^{-1}$; K_2HPO_4 $2 \text{ g} \cdot \text{L}^{-1}$; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ $0.2 \text{ g} \cdot \text{L}^{-1}$; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ $0.05 \text{ g} \cdot \text{L}^{-1}$; glucose $120 \text{ g} \cdot \text{L}^{-1}$. The time-course of lactic acid production in a batch fermentation on such a MRS medium is plotted in **Figure 34**. The cells were well adapted on this medium and lactic acid production began immediately after adding the inoculum. The process was terminated after about 20 hours. In comparison with that, the lactic acid production was retarded, when a MRS medium containing 50 % (v/v) of barley hydrolysate was used. The process ended after 36 hours due to the longer lag phase at beginning.

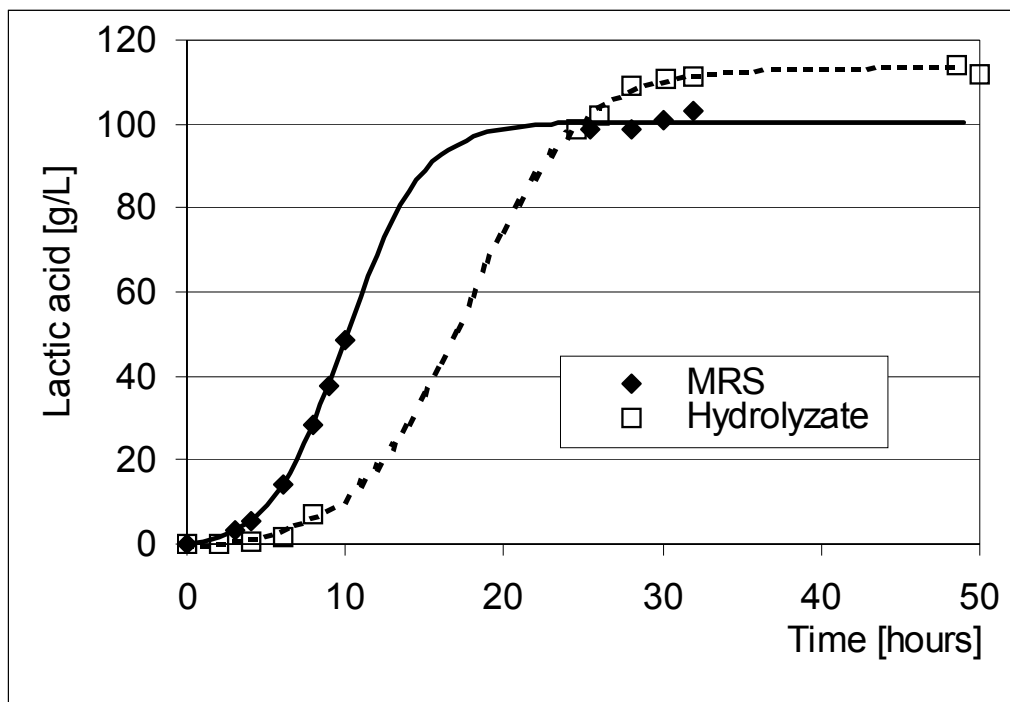


Figure 34: Lactic acid production obtained in discontinuous cultivation of *Lactobacillus paracasei* 168 on a complete MRS medium and a barley hydrolysate MRS medium at a temperature of 40.5°C and a pH value of 6.0

Initially, time functions describing exactly the change of the concentrations of the product P (lactic acid) are determined by model approximation. Again the sigmoidal function (equation 1)

$$P_{(t)} = a + \frac{b}{(1 + \exp(-(t - c) / d))}$$

was found to be suitable for use as a target function. This procedure could be achieved by means of the computer programme TableCurve 2D (Jandel Scientific Software).

The coefficients a, b, c, and d are constants which are calculated by the computer by iteration. The time functions for the most important parameter lactic acid obtained on the way described are presented in **Table 18** and **Figure 34** by lines respectively. The accuracy data ($r^2 = 0.999$ for both trials) supports the excellent model approximation attained using the sigmoidal equation.

Table 18: Comparison of the model parameter of lactic acid production

Parameter of equation (1)	MRS	Hydrolyzate
a	-1.631	-1.902
b	99.571	115.212
c	10.090	17.457
d	2.388	3.611

The second step is to form the first derivation dP/dt of the above mentioned logistic equation and obtain through this the time course of the volumetric production rates for the product P. All of these curves run through maxima representing the maximal productivities of lactate production, which may be achieved by the strain tested under the conditions of cultivation tested.

The differences in lactate productivity of the strain No. 168 for fermentation runs in a complete rich nutrient medium and a blended medium with barley hydrolyzate are plotted in **Figure 35**.

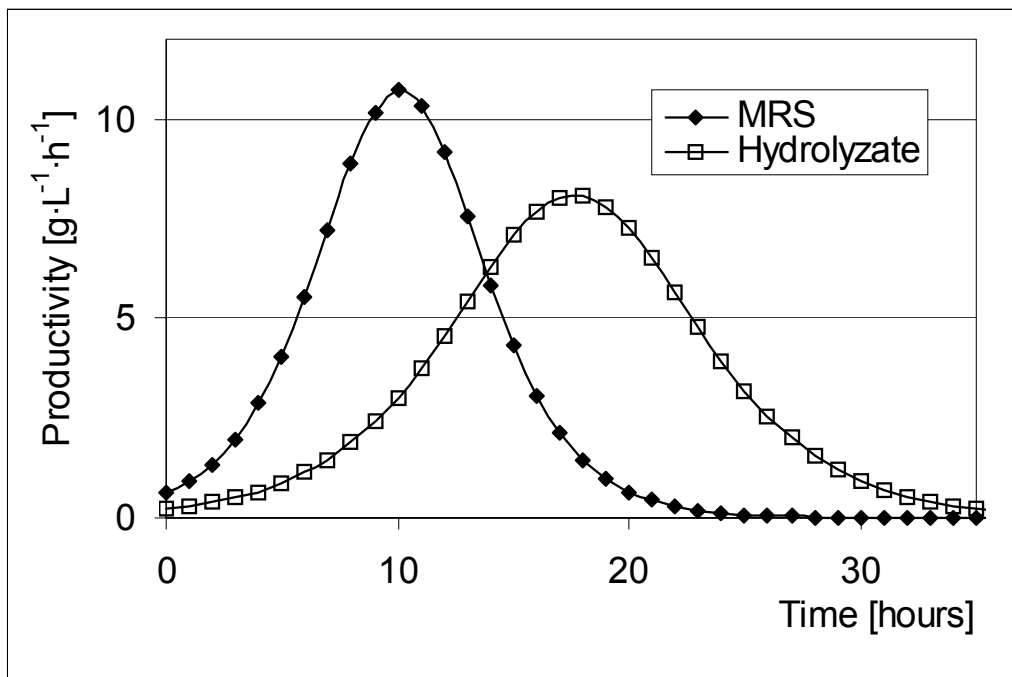


Figure 35: Productivity obtained in discontinuous cultivation of *Lactobacillus paracasei* 168 on a complete MRS medium and a barley hydrolysate MRS medium at a temperature of 40.5°C and a pH value of 6.0

The maximum of lactate productivity on a blended medium is at a lower level and appears later in comparison to the full nutrient MRS medium. That means the microorganisms need a little bit longer to adapt to the complex medium barley and the concentration of the nutrients is not so optimal like for the long-term used, well reliable MRS medium.

5.3 Batch cultivation of a lactic acid bacterium on lucerne green juice

The next step was to test the green material as a nutrient supplement instead of any other expensive nitrogen sources. Firstly it was to investigate whether the green juice contains unknown substances which might be interfering the bacterial growth.

The different time courses of lactic acid concentration and the productivity for the three comparable fermentation runs are presented in **Figure 36**. The results for the green

juice of lucerne indicate a slower microbial growth and product formation respectively. There is also a delay in the development of the maximum productivity and a lower number of that parameter. But there are no negative influences on the fermentation process in general and the final lactate concentration is in the same range as for the other ones.

A series of experiments is necessary to test the suitability of the green juice and the protein concentrate to substitute the present nitrogen and protein sources yeast extract, peptone and meat extract.

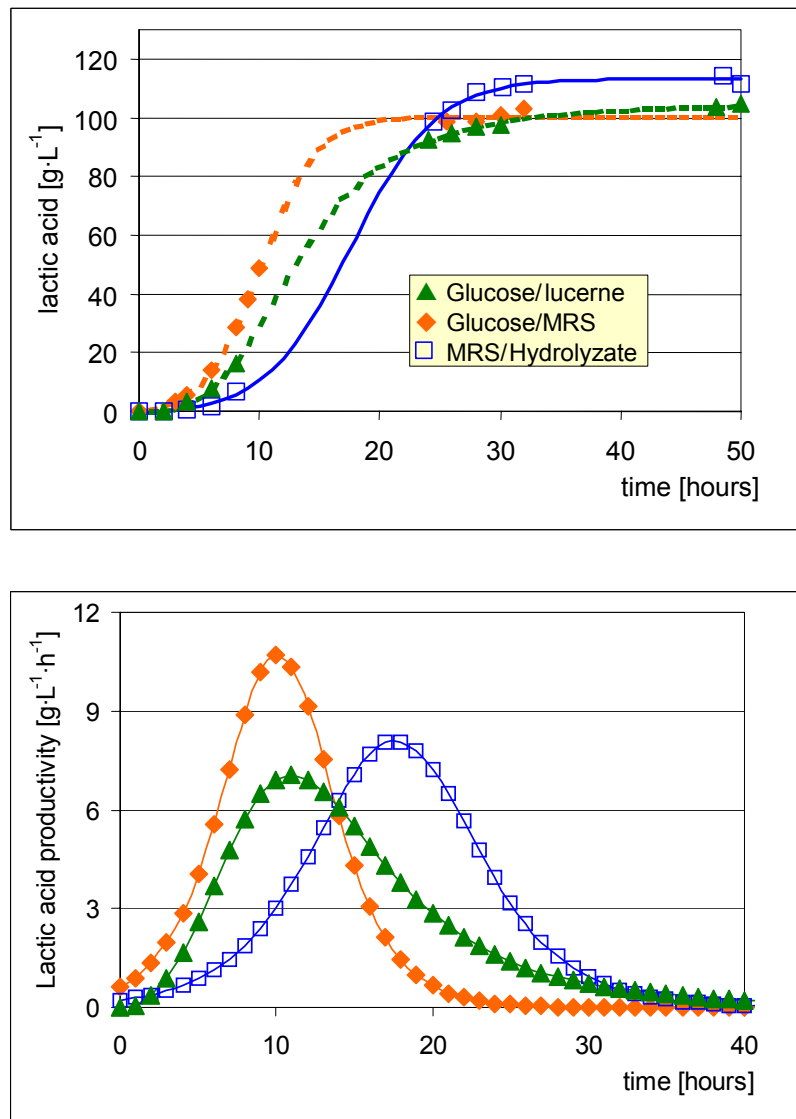


Figura 36: Product formation of the strain No. 168 on different nutrient media

The final concentration of lactic acid is in the same range for all fermentation runs (**Figure 37**). Considering the partial different glucose concentration the yield of lactic acid (product concentration in relation to the used substrate concentration) shows slightly higher values for the experiments with barley hydrolyzate and green juice respectively.

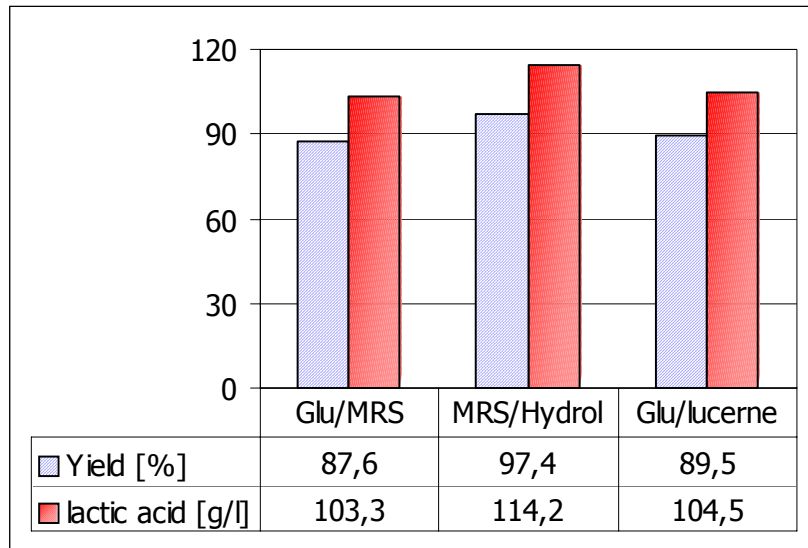


Figure 37: Yield and final concentration of lactic acid under different conditions

Up to now it can be summarized that the utilization of barley hydrolysate and green juice can substitute the main part of the nutrient medium for lactic acid bacteria.

This positive result for the utilization of different renewable resources in lactic acid fermentation has to be confirmed and optimized. In addition to that the next experiments will include material from Iceland (barley, lupine).

The different time courses of lactic acid concentration and the productivity for three comparable fermentation (barley hydrolysate from Iceland together with lupine juice) runs are presented in **Figure 38 and 39**.

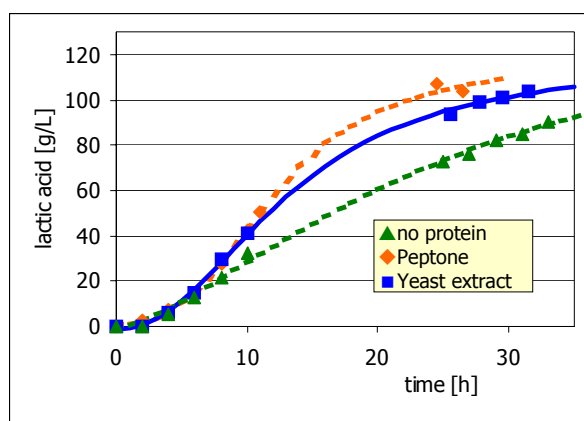


Figure 38: Time course of lactic acid with different nitrogen sources

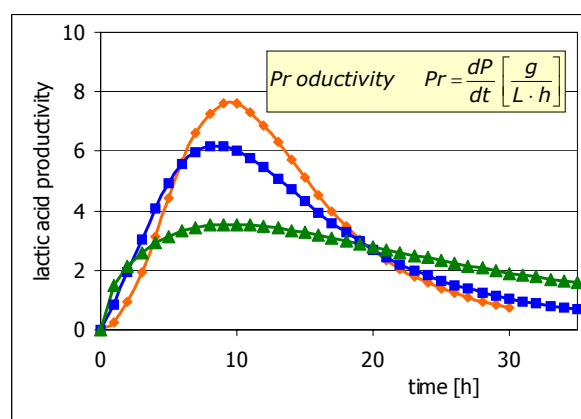


Figure 39: Fitting of the calculated lactic acid productivity

The results for the addition of peptone indicate the best product formation whereas there is only the half of the maximum productivity without any additional proteins.

5.4 Lactic acid production in bench scale

This research was continued in pilot scale with different raw materials as feedstocks (barley, rye) to find out the differences between the plants as starch/glucose sources for hydrolysis and fermentation. The separation (ultrafiltration) of the glucose solution after hydrolysis for the fermentation process was more difficult for rye in comparison to barley. The hydrolysate of rye contains some slimy substances (glucans, pentosans) which have negative effects to the permeability of the used membranes for filtration. Therefore the yield of glucose from rye (considering the whole process of hydrolysis and ultrafiltration) is lower than for barley.

Batch fermentation runs of the strain *Lactobacillus paracasei* 168 were carried out in a 75-litre-bioreactor on nutrient-enriched rye and barley hydrolysate medium respectively (**Figure 40**). The addition of nutrients happened with respect to the previous laboratory investigations, e.g. reduced concentration of salts due to the natural content of corn hydrolysate. The yields of lactic acid were nearly 100 % relating to the starting concentration of glucose.

After hydrolysis there is mostly a residual part of disaccharides in the liquid which can be converted by the microorganisms additional to the glucose content. The productivity for lactic acid was calculated to a range between 3 and 5 g · L⁻¹ · h⁻¹.

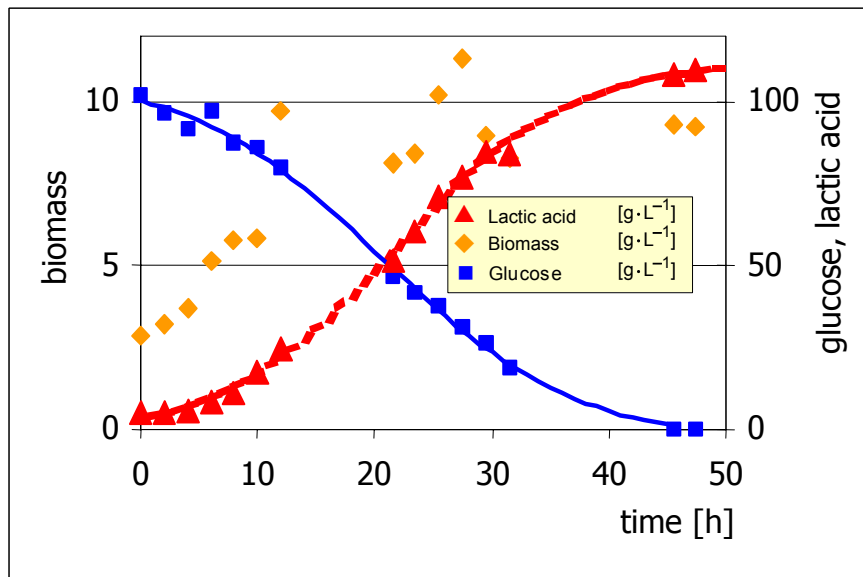


Figure 40: Time course of lactic acid fermentation on barley hydrolysate

6 Continuous lactic acid fermentation process

6.1 Operation in chemostat mode

After the optimization of the parameters pH-value, temperature and the composition of the fermentation medium respectively the process operation were changed firstly from a discontinuous (batch) to a fed-batch and finally to a continuous process. The fermentation runs have been carried out with barley hydrolysate from Germany with reference to the results from the previous batch experiments. At the beginning of the continuous cultivation there were tested several settings of the input flow for different nutrients (carbon source, salts). Depending on the activity of the lactic acid bacteria the overall dilution rate D changed (0.03 up to 0.1 h^{-1}) due to the demand of base to maintain the pH at the optimal value. At the steady state of a continuous fermentation process the specific growth rate equals the dilution rate. The next **Figure 41** illustrates a typical time course of a continuous fermentation process in chemostat mode (without cell retention).

For an overall dilution rate of about 0.1 h^{-1} the biomass concentration is very low and the lactic acid concentration decreases in correlation with the cell number. After 65 hours the number of living cells increases up to a range between 10^8 up to 10^9 cells/mL. At the same time the lactic acid concentration was stabilized at a nearly constant level. It seems that the cell number of living microorganisms is the key factor for yield and productivity.

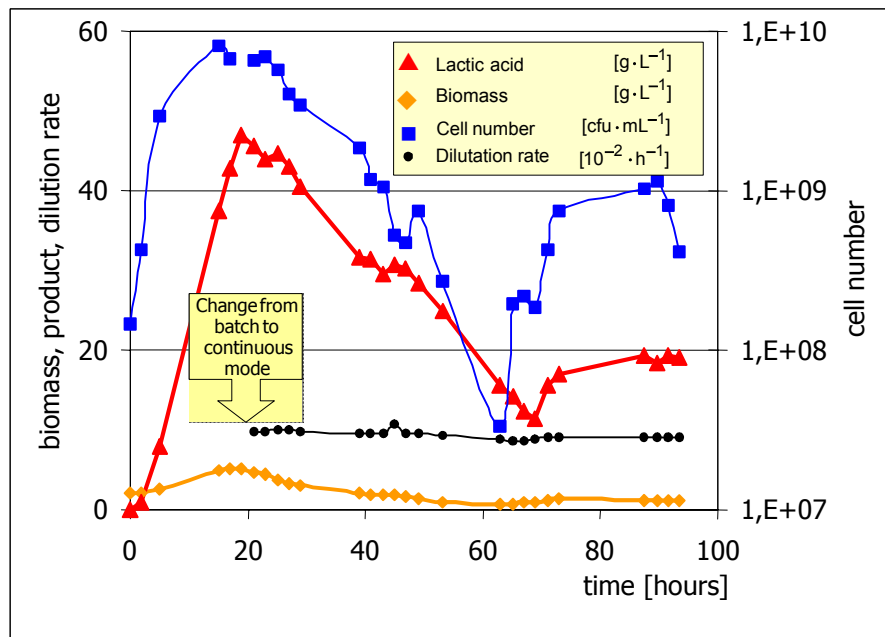


Figure 41: Typical time course of a lactic acid fermentation in chemostat mode without cell recycle

6.2 Cell recycle bioreactor

Due to the continuous loss of viable microorganisms there is a limit of the lactic acid productivity Pr , which is defined as the product of the dilution rate D and the lactic acid concentration P :

$$Pr = D \cdot P \quad (16)$$

To increase the overall yield of the whole fermentation process it is necessary to enhance whether one or both of the above mentioned parameters. Depending on the input concentration of the main substrate (glucose content of the hydrolysate) the lactic acid bacteria need a certain time (residence) to degrade the carbon source nearly completely. In respect to the phenomenon of product inhibition on the one hand and substrate limitation on the other hand there is a need to optimize the productivity.

Therefore an important parameter to improve the lactic acid production seems to be the amount of active biomass (cfu – colony forming units) in the bioreactor. One of the usual ways to keep the biomass inside of the system is the cell retention with hollow fibre membranes. The basically set-up of such a combination consisting of a bioreactor and filtration module is illustrated in **Figure 42**.

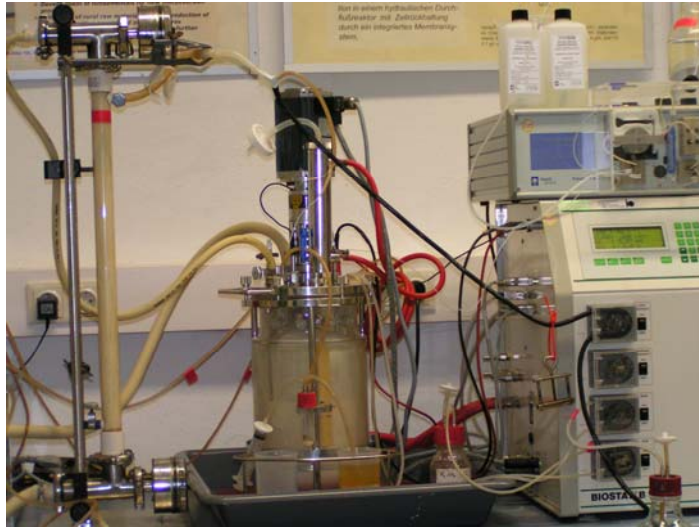


Figure 42: Set-up of a continuous lactic acid fermentation with cell retention by a hollow fibre module

It can be seen very clearly from the **Figure 43** the effect of cell retention in respect to the same parameters like in the previous example of a chemostat fermentation in **Figure 41**. There is only a slight decrease of the lactic acid concentration which is affected by the composition of the fermentor input. The amount of living cells increases all the time up to a range between 10^{10} up to 10^{11} cells/mL (please note the different scaling of both y-axes!) in correlation to the growth of biomass.

According the equation for the calculation of the productivity this important characteristic value of a continuous fermentation process could be increased up to $25 \text{ g} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$. In comparison to the process without cell recycle there is a triple up to four times higher productivity of lactic acid.

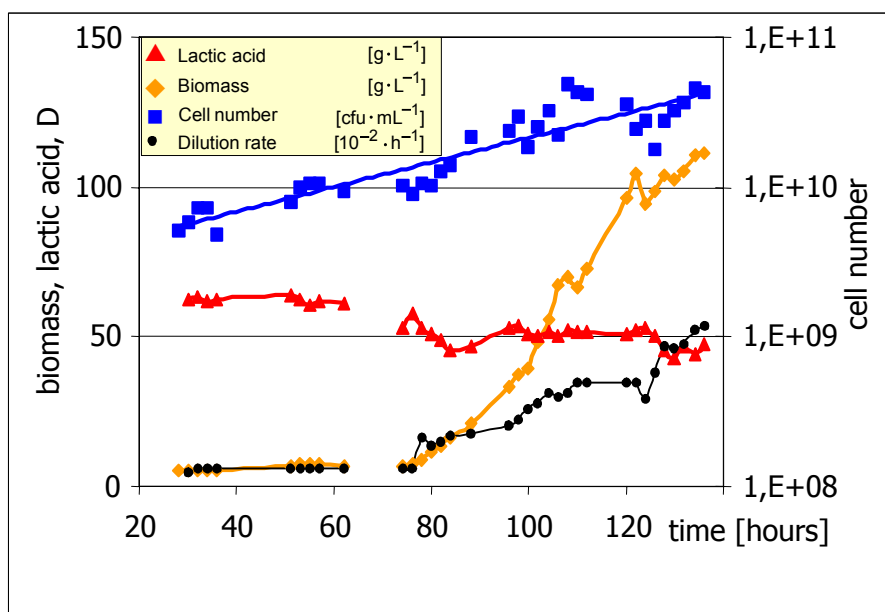


Figure 43: Typical time course of a lactic acid fermentation with cell retention by a hollow fibre module

6.3 Conclusions

The strain *Lactobacillus paracasei* 168 was selected to be the most appropriate by means of different methods and will be the basis for developing fermentation technology. It was found that the new strain is able to accumulate more than 100 g of lactic acid per litre in the medium and the yield of lactic acid can be higher than 90 %. The process parameters pH value and temperature for pure nutrients were fixed at 6.0 and 40.5°C respectively as a compromise of different aims (productivity, yield, duration).

In view of preparing a basic nutrient for the fermentation of lactic acid bacteria one of the most important parameters was the accessible concentration of glucose after treatment of the starch containing corn. According to the intention to substitute expensive nutrient supplements by cheaper materials from renewable resources there were tested green material from lucerne and lupine for the cultivation of lactic acid bacteria. The green juice after pressing fresh biomass contains a series of nitrogen-containing compounds and anorganic salts, which are essential for cell growth.

The objective was to substitute the expensive yeast and meat extracts as well as peptone by the proteins containing in the green juice. There were more than 45 runs of laboratory fermentations based on the different renewable materials mentioned above. Up to now it can be summarized that the utilization of corn hydrolysate and green juice can substitute the main part of the nutrient medium for lactic acid bacteria.

This research was continued in pilot scale with different raw materials as feed-stocks (barley, rye) to find out the differences between the plants as starch/glucose sources for hydrolysis and fermentation. The addition of nutrients happened with respect to the previous laboratory investigations, e.g. reduced concentration of salts due to the natural content of corn hydrolysate.

After the optimization of the parameters pH-value, temperature and the composition of the fermentation medium respectively the process operation were changed firstly from a discontinuous to a fed-batch and finally to a continuous process. For the continuous cultivation there were tested several settings of the input flow for different nutrients (carbon source, salts), procedures of cell retention and control systems.

The effect of the single down-streaming processes with respect to product concentration, nutrients and several ions respectively have been investigated. Depending on the utilization of the lactic acid and the further processing to manufacture other products like ethyl lactate the demand of purity has to be defined. The main achievement is the development of a basic continuous lactic acid fermentation process with cell retention and control of the glucose concentration with a significant enhancement of the yield of lactic acid and biomass respectively by cell retention (up to 400 % higher productivity!).

7 Down-stream processing of lactic acid

The process steps required to gain the final product after fermentation are termed down streaming. Depending upon the purity requirements, several process steps are combined that exert a significant influence on the costs. The expenses for the down streaming of a bio-product, for example, amount to up to 90 % of the entire production costs, depending on the value of the product (KASCHE 2000). Therefore the down streaming of fermentation media is of particular importance.

A complete production scheme, targeted around membrane operations for clarification, concentration and conversion of sodium lactate after fermentation, was investigated by (BAILLY *et al.* 2001). The fermentation broth is first clarified by cross flow microfiltration from biomass. This is required to obtain a fluid with appropriate properties for carrying out electrodialysis in proper conditions. After conversion of sodium lactate into lactic acid by EDB, the product is finally upgraded by ion exchange resins, whereby polluted effluents of rinsing water and consumption of regenerants are minimized.

7.1 Methods

7.1.1 Membrane filtration

The membrane filtration studies were carried out in a pilot plant of Messrs. UFI-TEC GmbH Oranienburg (Germany) with exchangeable modules and membranes as shown in **Figure 44**.

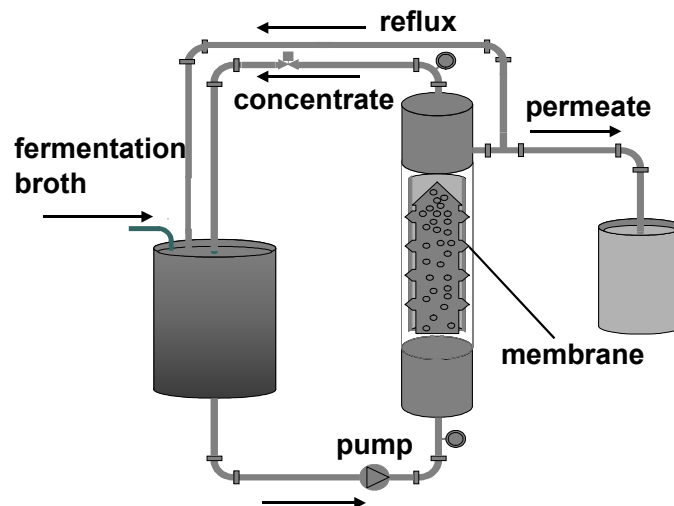


Figure 44: Test equipment for membrane filtration

The temperature was kept constant at 35°C by using a heat exchanger. In the tubular module used, capillary membranes with a membrane effective filtration area of 0.1 m² were applied.

The characteristic values of the membrane UF-MD 020 UP-3N (MICRODYN Wuppertal, Germany) examined are:

material	PES
number of capillaries	48
inner diameter	1.5 mm
pore size	30 nm

Samples were drawn according to a predetermined schedule for analysis of different ingredients. The temperature, the pressure in front and behind the membrane, as well as the permeate flow were registered regularly during the experiments.

7.1.2 Removal of divalent cations

The laboratory scale IE unit had a column, which was filled with the ion exchange resin. The column with frit (diameter of pores: 250 - 500 μm) from QVF Labortechnik Ilmenau, Germany, was 1750 mm high with an inner diameter of 22 mm. The bed volume of the resin was 0.5 L. Chelating resin RCH 46 (Eurodia Industrie SA, Pfullingen, Germany) was used to remove multivalent metal ions from clarified fermentation broth before the electrodialysis experiments. On the basis of the producer's specification, the following separation and regeneration procedure was applied:

Separation

1. Down-flow: 12.6 bed volumes of sodium lactate solution. Flow rate: $3 \text{ L} \cdot \text{h}^{-1}$.

Regeneration

1. Down-flow: rinsing with 6 bed volumes of de-ionized water. Flow rate: $3 \text{ L} \cdot \text{h}^{-1}$.
2. Up-flow: rinsing with 4 bed volumes of de-ionized water. Flow rate: $4 \text{ L} \cdot \text{h}^{-1}$.
3. Down-flow: regeneration with 3 bed volumes of HCl ($40 \text{ g} \cdot \text{L}^{-1}$). Flow rate: $3 \text{ L} \cdot \text{h}^{-1}$.
4. Down-flow: slow rinsing with 4 bed volumes of de-ionized water. Flow rate: $2 \text{ L} \cdot \text{h}^{-1}$.
5. Down-flow: fast rinsing with 4 bed volumes of de-ionized water. Flow rate: $4 \text{ L} \cdot \text{h}^{-1}$.
6. Up-flow: regeneration with 2 bed volumes of NaOH ($40 \text{ g} \cdot \text{L}^{-1}$). Flow rate: $2 \text{ L} \cdot \text{h}^{-1}$.
7. Up-flow: slow rinsing with 4 bed volumes of de-ionized water. Flow rate: $2 \text{ L} \cdot \text{h}^{-1}$.
8. Down-flow: fast rinsing with 4 bed volumes of de-ionized water. Flow rate: $4 \text{ L} \cdot \text{h}^{-1}$.

Samples were drawn from the original solution at the beginning of a run, in accordance with a predetermined schedule, and at the end of a run for analysis of lactic acid and ions of Ca and Mg.

7.1.3 Electrodialysis

The electrodialysis experiments for mono-polar and bipolar electrodialysis were conducted using a laboratory facility with 4 cycles (**Figure 45**) and the membrane stack OS-ED-100 Quadro (OSMOTA Membrantechnik GmbH Rutesheim, Germany). The membranes ACS/CMS were used for the mono-polar electrodialysis and the mono-polar membranes AMX/CMX as well as the bipolar membranes of Neosepta, Tokuyama Corp., Japan, for the bipolar electrodialysis. The size of the cell frame was 15 x 15 cm with a thickness of 0.5 mm. The effective membrane area was 100 cm². The number of membranes used comprised 10 AMX-, 11 CMX- and 10 bipolar membranes. Each circuit was equipped with a pump and measuring devices for flow, pressure, temperature, pH-value and conductivity. Conductivity, pH and temperature were measured with a MultiLine P3pH/LF (WTW Weilheim, Germany). The power supply to the membrane stack was provided via a direct current supply unit (EA-PS 7065 – 10 A, EA Elektro-Automatik GmbH Viersen, Germany) with a controllable voltage from 0 to 50 V and current from 0 to 10 A, designed for running with constant current and constant voltage.

The experiments were carried out in a batch mode. Four storage tanks (each 10 L) were used to hold dilute (salt), concentrated (acid), base, and electrode rinse solutions. The stock tanks were temperature-managed by thermostat to maintain the temperature between 33 and 36°C. In all stack configurations, the electrode rinse solution was recirculated continuously to transfer the electric current and to remove gases produced by the electrode reaction during the operation of the ED. From each chamber, 10 cm³ samples were taken according to a predetermined schedule for analysis of lactic acid, inorganic and organic materials.

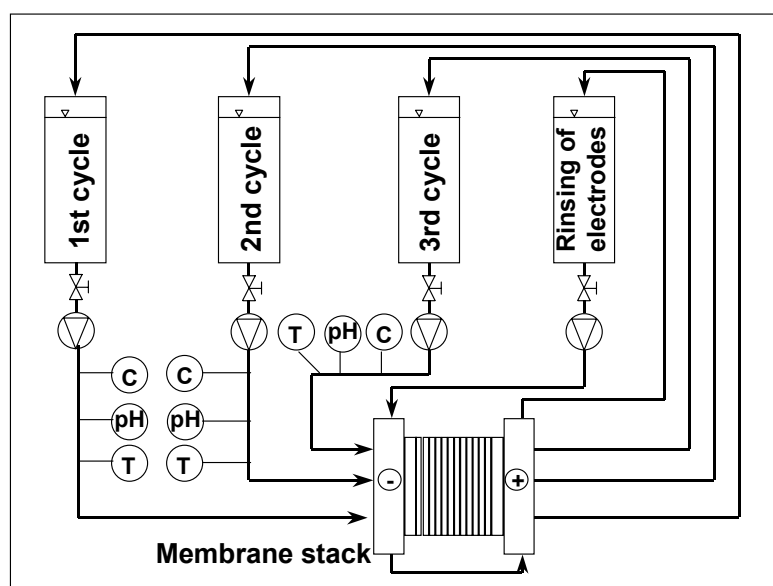


Figure 45: Apparatus for electrodialysis experiments

7.1.4 Mono-polar electro dialysis

The principle is illustrated in **Figure 46**.

The electrode rinse solution ($\text{Na}_2\text{SO}_4 - 5 \text{ g} \cdot \text{L}^{-1}$), the concentrate (sodium lactate – initial concentration $6.6 \text{ g} \cdot \text{L}^{-1}$) and the diluate (sodium lactate – initial concentration $115 \text{ g} \cdot \text{L}^{-1}$) were circulated through the corresponding chambers of the stack with the flow rates in the three channels ranging from 2.5 to $3.0 \text{ L} \cdot \text{min}^{-1}$ for each chamber. For the period of constant voltage 20 V was applied. The experiments were finished when the conductivity in the diluate dropped to $8.0 - 9.5 \text{ mS} \cdot \text{cm}^{-1}$ (concentration of sodium lactate 8 to $10 \text{ g} \cdot \text{L}^{-1}$).

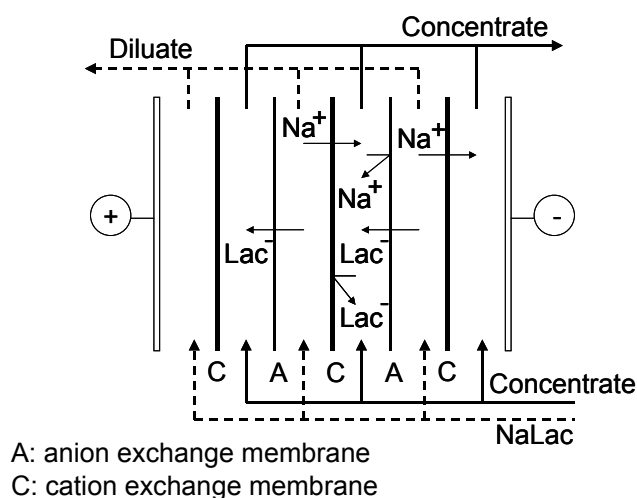


Figure 46: The process scheme of mono-polar electro dialysis

7.1.5 Bipolar electro dialysis

The following solutions were used for the EDB: $\text{NaOH} - 9.5 \text{ g} \cdot \text{L}^{-1}$ (electrode rinse solution), $\text{NaOH} - 9.5 \text{ g} \cdot \text{L}^{-1}$ (base stream), lactic acid – $5 \text{ g} \cdot \text{L}^{-1}$ (acid stream). A current density of $500 \text{ A} \cdot \text{m}^{-2}$ was applied. The circulation flow rates in the channels ranged from 2.5 to $3.0 \text{ L} \cdot \text{min}^{-1}$.

The three-compartment bipolar electro dialysis unit (EDB3C) consisted of ten cell pairs with bipolar membranes, anion exchange membranes and cation exchange membranes, and four solution tanks (salt stream, acid stream, base stream, and electrode rinse solution). Bipolar membranes, anion exchange membranes, and cation exchange membranes were arranged alternately. During ED operation lactate ions and sodium ions in the salt compartment moved simultaneously into the acid and base compartments through anion and cation exchange membranes, respectively. Free lactic acid was formed by combination of lactate ions and hydrogen ions generated on the cation exchange layer of the bipolar membrane, while sodium hydroxide was generated simultaneously by combination of sodium ions and hydroxyl ions on the anion exchange side of the bipolar membrane (**Figure 47**).

The two-compartment bipolar electro dialysis unit (EDB2C) consisted of ten cell pairs with bipolar membranes and cation exchange membranes, and three solution tanks (salt/acid stream, base stream, and electrode rinse solution). Bipolar membranes and cation exchange membranes were arranged alternately. The cation exchange membranes allow the selective passage of sodium ions from the salt solution, but reject lactate ions.

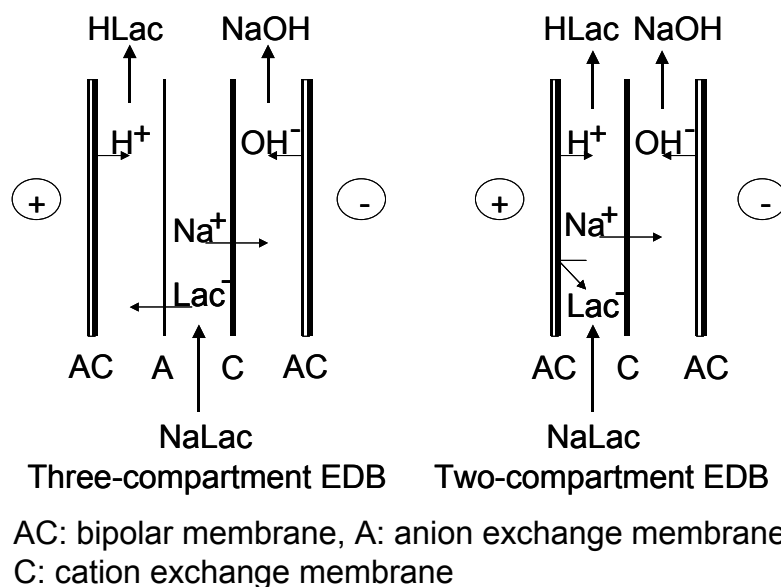


Figure 47: The process scheme of the three- and two-compartment bipolar electro dialysis EDB

7.1.6 Ion exchange

Ion exchange (IE) is a fixed-bed separation technology using ion exchange resins. These resins have a high number of firmly attached bonds on their surfaces, which can adsorb anions and cations reversibly. The capacity of exchangeable ions is limited, however. The quantity of ions that can be separated by given quantities of resins has to be determined experimentally. The same applies with regard to the required amount of rinsing water and regenerant, which removes the adsorbed ions and thus regenerates the resin to its previous state. The ion exchange resins Relite EXC08 (strongly acidic resin) and Relite EXA133 (weakly basic resin) from Residion S.R.L. Mitsubishi Chemical Corporation, Italy were used.

7.1.7 Analytical Methods

Lactic acid and glucose concentrations were measured by HPLC using a GYNKOTEK chromatograph, Germany (column: Eurokat H (KNAUER); 300 · 7.8 mm I.D.; eluent: 0.003 n H₂SO₄; flow rate: 0.8 mL · min⁻¹; sample volume: 50 μL; temperature: 25°C; pressure: 3 MPa; detection: RI). The concentration of sodium lactate was calculated from the concentration of lactic acid. Total Kjeldahl nitrogen (TKN) was analyzed using standard-method Vapodest apparatus from Gerhardt by digestion using a selenium

catalyst. The colorimetric technique was used to measure total phosphorus (TP) with the molybdenum blue method. Anions and cations were determined under the following conditions using the ion chromatograph DX-120 from Dionex, Idstein: column: IonPac AS14 (4 mm) with precolumn (anions); Ion-Pac CS12A (4 mm) with precolumn (cations); eluent: 3.5 mM disodium carbonate, 1 mM sodium hydrogen carbonate (anions), 22 mM sulfuric acid (cations); flow rate: 1.12 mL · min⁻¹ (anions), 1.1 mL · min⁻¹ (cations); detection: conductivity with auto-suppression; suppressor: ASRS in the recycle mode (anions), CSRS in the recycle mode (cations); injection volume 25 µL; elution duration: 12 min (anions), 14 min (cations). Concentrations of Ca- and Mg-Ions were determined by AAS (Vario 6, analytikjena, Germany).

7.2 Results and Discussion

7.2.1 Ultrafiltration

The components of sodium lactate solution after fermentation are set out in **Table 19**. The fermentation broth from the lactic acid fermentation was filtered to remove the cells in order to prevent deposition of bacteria on the membrane surface and the creation of bacteria clusters in the space between the membranes of the electrodialysis unit. All cells of the fermentation broth were removed and cell-free permeate was obtained by ultrafiltration (UF).

Table 19: Components of sodium lactate solution after fermentation

Components	Unit	Value
Sodium lactate	(mg · L ⁻¹)	130700
Glucose	(mg · L ⁻¹)	1690
Biological dry solid matter BTS	(mg · L ⁻¹)	9300
Total Kjeldahl nitrogen TKN	(mg · L ⁻¹)	1670
total phosphorus TP	(mg · L ⁻¹)	607
Na ⁺	(mg · L ⁻¹)	33100
K ⁺	(mg · L ⁻¹)	1520
Ca ²⁺	(mg · L ⁻¹)	58
Mg ²⁺	(mg · L ⁻¹)	159
NH ₄ ⁺	(mg · L ⁻¹)	97
Cl ⁻	(mg · L ⁻¹)	588
PO ₄ ³⁻	(mg · L ⁻¹)	526
SO ₄ ²⁻	(mg · L ⁻¹)	2160
NO ₂ ⁻	(mg · L ⁻¹)	< 5
NO ₃ ⁻	(mg · L ⁻¹)	< 5
pH	-	6.49
Conductivity	(mS · cm ⁻¹)	44.6
Extinction	(420 nm)	3.5

Owing to deposition of organic and inorganic substances on the membrane surface and in its pores (fouling and scaling), permeate flux of UF-membranes decreased within the first two hours. After this period further deposition was prevented by the overflow velocity of the solution. The concentration factor amounted to 19.7, equivalent to a permeate recovery rate of 95 %. By rejection of biomass the concentrations of different compounds are changed, as shown in **Table 20**. Sodium lactate was able to pass through the membrane completely.

Table 20: Composition of initial and treated fermentation broth by ultrafiltration

Compound	Initial fermentation broth	Treated fermentation broth	Rejection (%)
Sodium lactate (mg · L ⁻¹)	130700	130700	0
BTS (mg · L ⁻¹)	9300	0	100
TKN (mg · L ⁻¹)	1670	1130	32
TP (mg · L ⁻¹)	607	527	13

7.2.2 Removal of multivalent metals

Multivalent metal ions (Ca, Mg etc.) have to be removed from the cell-free fermentation broth. Their concentration must be altogether lower than 1 mg · L⁻¹.

According to the specification of the chelating resin RCH 46, the total exchange capacity of 1 L resin corresponds to 1 equivalent cation of heavy metals. The capacity of this resin is dependent upon the pH (alkaline pH for Mg and Ca). It is recommended that laboratory trials (column tests) be carried out to prove the process. Since the resin used was new, after preliminary tests the process was designed with 20 % of the determined capacity.

These data and concentrations of Ca and Mg ions in the solution of sodium lactate resulted in a capacity of 6.2 L solution/BV resin. The solution was separated at a flow rate of 6 BV/hr. To obtain a high yield of sodium lactate in the collected solution, approximately 0.68 BV of rinsing water were necessary. The total loss of sodium lactate was 5.8 %. The rejection for Ca- and Mg-ions was 99.1 % and 99.6 %, respectively (**Table 21**).

Table 21: Composition of initial and treated sodium lactate solution using chelating resin RCH 46

Compound	Cell free fermentation broth	Treated fermentation broth	Rejection (%)
Sodium lactate (mg · L ⁻¹)	130700	115200	11.8
Ca ²⁺ (mg · L ⁻¹)	58	0.5	99.1
Mg ²⁺ (mg · L ⁻¹)	159	0.1	99.9

It is possible to control the procedure by measuring the conductivity and the pH-value of the solution after passing the column (**Figure 48**). The breakthrough of dissolved sodium lactate and starting point of rinsing are marked with an arrow. The sudden increase in conductivity and the sudden decrease in the pH-value mark the breakthrough and the beginning of ion exchange. Finally, the solution displaced by the rinsing solution in the column is diluted with a part of the bed volume of the rinsing solution. As a result, the concentration of sodium lactate in the solution decreases from $130.7 \text{ g} \cdot \text{L}^{-1}$ to $115.2 \text{ g} \cdot \text{L}^{-1}$ after processing through the column. According to the specification of 1 BV resin, 60 g HCl (pure), 40 g NaOH (pure), and 26 BV de-ionized water for rinsing were needed for the regeneration and rinsing step of a run.

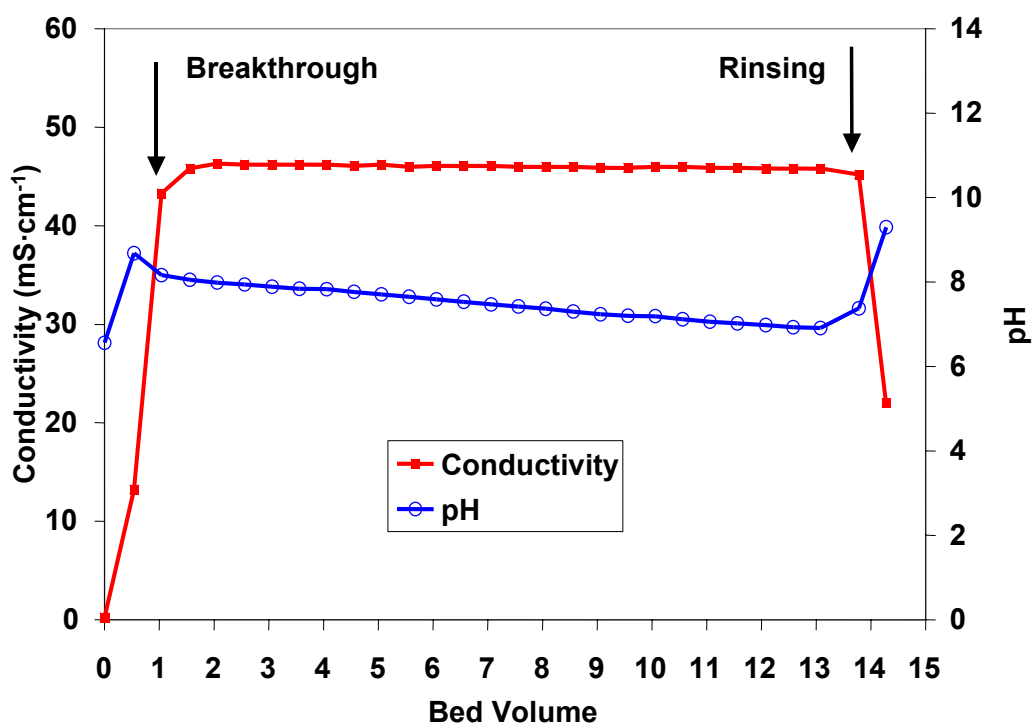


Figure 48: Conductivity and pH-value plotted as a function of throughput volumes measured in units of bed volumes using chelating resin RCH 46

7.2.3 Conventional electrodialysis (EDM)

The product sodium lactate is concentrated and purified by conventional EDM electrodialysis. The value of the concentration is a very important factor for the subsequent lactic acid recovery step of electro-conversion. The degree of concentration (the ratio of the final concentration in the concentrate to the initial diluate concentration) can be influenced by the ratio of the initial diluate volume to the initial concentrate volume. For the trials the degree of concentration was limited by a minimal value of 1 L in the concentrate. The electrodialysis was therefore operated in two-level mode.

Performance parameters for two-level electrodialysis are listed in **Table 22** and show that an increase of sodium lactate in the concentrate by two-level electrodialysis does

not affect water transport, current efficiency or specific energy consumption considerably.

Since only charged particles are transported in the electric field during the electro dialysis, purification was carried out for the concentrate solution of uncharged particles. This led to glucose and uncharged compounds of nitrogen remaining in the diluate solution (**Table 23**).

Table 22: Parameter during two-level conventional electro dialysis of sodium lactate solution

Parameter	Stage of experiment	
	1 st stage	2 nd stage
Operating time (hr)	17	15.33
Voltage (V)	20	20
Current density (A/m ²)	140	130
Initial volume of diluate (L)	8	7
Initial volume of concentrate (L)	1	3.677
Initial concentration of diluate (g · L ⁻¹)	115.2	115.2
Final concentration of diluate (g · L ⁻¹)	8	10
Initial concentration of concentrate (g · L ⁻¹)	6.6	209
Final concentration of concentrate (g · L ⁻¹)	209	236
Water transport (L/eq)	0.39	0.4
Current efficiency (%)	76	78
Specific energy consumption (kWh/kg)	0.63	0.62

Table 23: Concentrations of selected ingredients in the diluate and concentrate solution after two-level conventional electro dialysis of sodium lactate solution

Compound	Diluate solution	Concentrate solution	Rejection (%)
Sodium lactate (mg · L ⁻¹)	115200	236000	-
Glucose (mg · L ⁻¹)	1500	0	100
TKN (mg · L ⁻¹)	1000	389	61

7.2.4 Bipolar electro dialysis (EDB)

The bipolar electro dialysis was investigated using a two- and three-compartment membrane stack configuration. For the first configuration the two-compartment stack consisted of base and salt compartments and was assembled using alternating bipolar and cation exchange membranes. Anion exchange membranes were not used because of their short lifetime. The three-compartment membrane stack consisted of anion exchange, cation exchange and bipolar membranes to allow three streams to flow, i.e. acid, base, and salt streams.

7.2.4.1 Two-compartment bipolar electro dialysis (EDB2C)

The experiment was carried out in a constant current mode (5 A). **Figure 49** shows the time course of the EDB2C. The trial was stopped when, after a decrease, the electrical conductivity of the salt-/acid-stream increased again. A final lactic acid concentration of about $188 \text{ g} \cdot \text{L}^{-1}$, corresponding to 95 % conversion, was obtained. The specific energy consumption for the recovery of 1 kg of lactic acid was 1 kWh and the current efficiency was 57 %.

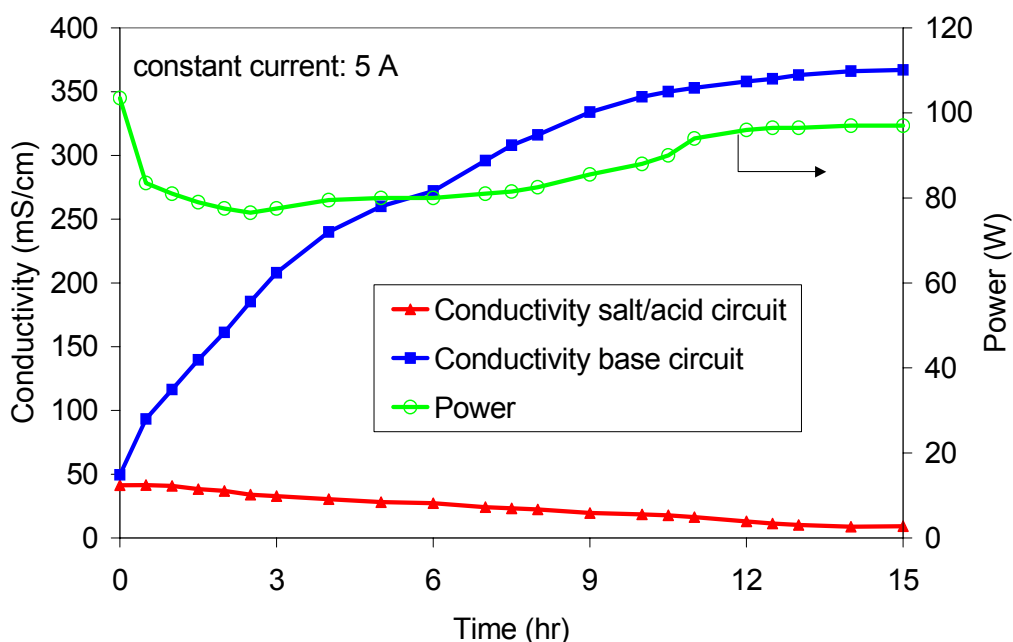


Figure 49: Evolution of conductivity in the salt/acid and base circuit as well as of power vs. time during the two-compartment bipolar electro dialysis of solution of sodium lactate

The purity of the lactic acid and sodium hydroxide produced is determined by the amount of organic and inorganic compounds in the recovered acid and base solution. In two-compartment bipolar electro dialysis, only cations from the salt solution can pass the cation exchange membranes (**Table 24**).

Table 24: Concentrations of selected ingredients in the sodium lactate solution and in the lactic acid solution after two-compartment and three-compartment bipolar electro dialysis (EDB)

Compound	Sodium lactate solution ($\text{mg} \cdot \text{L}^{-1}$)	Lactic acid solution		Rejection	
		Two-compartment EDB ($\text{mg} \cdot \text{L}^{-1}$)	Three-compartment EDB ($\text{mg} \cdot \text{L}^{-1}$)	Two-compartment EDB (%)	Three-compartment EDB (%)
TKN	389	269	81	31	79
K^+	2500	108	26	96	99
NH_4^+	88	1.4	7	98	92
Cl^-	886	855	851	4	4

7.2.4.2 Three-compartment bipolar electro dialysis (EDB3C)

The experiment was carried out in a constant current mode (5 A) like two-compartment electro dialysis. **Figure 50** shows the time course of the EDB3C. The trial was stopped at an electrical conductivity of 5 mS/cm in the salt solution. Experimental results are summarized in table 6. A final lactic acid concentration of about $183 \text{ g} \cdot \text{L}^{-1}$, corresponding to 95 % conversion was obtained. The specific energy consumption for the recovery of 1 kg of lactic acid was 1.24 kWh and the current efficiency was 73 %.

By comparison with three-compartment electro dialysis, the two-compartment electro dialysis has a much lower water transport, a lower current efficiency and a lower specific energy consumption for the same rate of conversion. By contrast, higher purity of the lactic acid is obtained with three-compartment electro dialysis (**Table 24**). Consequently the EDB2C is unsuitable for purification of lactic acid.

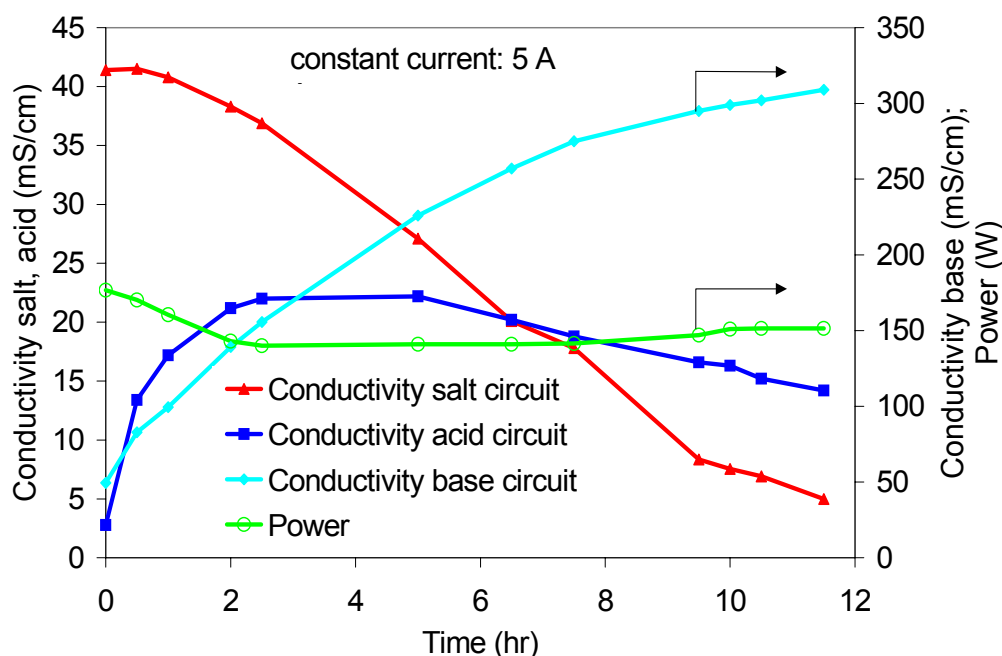


Figure 50: Evolution of conductivity in the salt, acid and base circuit as well as of power vs. time during three-compartment bipolar electro dialysis of solution of sodium lactate

7.2.5 Final purification stages.

Impurities of inorganic and organic compounds, which still remain in the concentrated lactic acid after both electro dialysis steps (mono- and bipolar), were almost completely eliminated by anion and cation exchange resins. The course of the conductivity and pH-value dependence on the throughput volumes is shown in **Figure 51**. The start and the end of the ion exchange (“Breakthrough” and “Rinsing” in figure 51) are indicated in each case by a change of conductivity and pH-value. Decolorisation of the lactic acid occurred at the same time in the cation interchanger.

The results listed in **Table 25** were achieved after concentration of the purified lactic acid by vacuum evaporation.

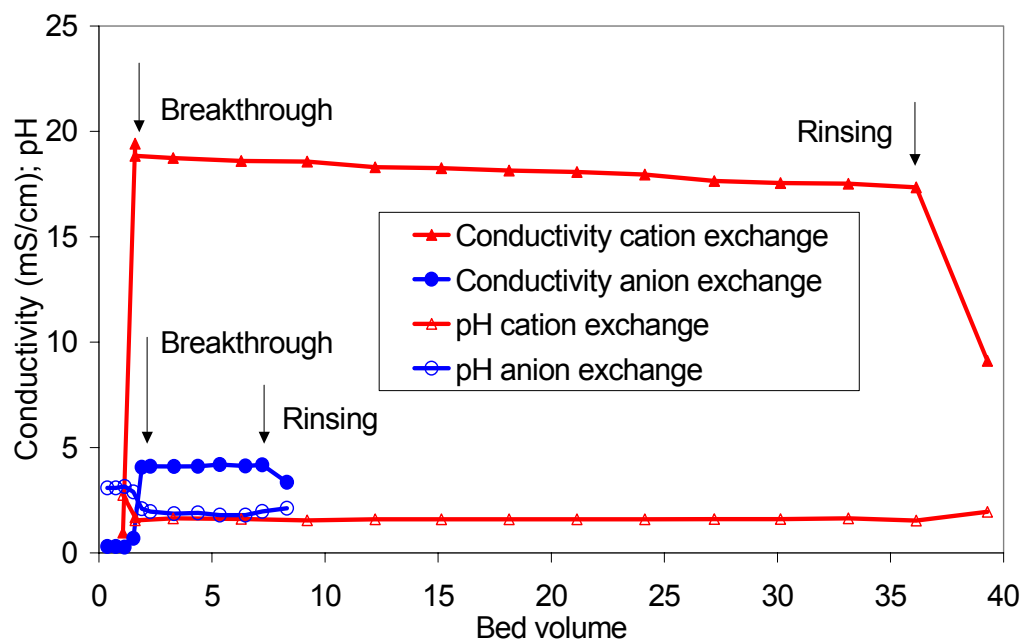


Figure 51: Conductivity and pH-value plotted as a function of throughput volumes measured in units of bed volumes by the up-flow desalting step for the anion and cation exchange

Table 25: Concentrations of selected ingredients in the lactic acid solution before and after desalting by cation and anion exchange resin as well as concentration by evaporation

Compound	Initial concentration	Final concentration	Rejection (%)
Hlac (mg · L ⁻¹)	183 200	668 400	-
Na ⁺ (mg · L ⁻¹)	359	18.4	95
K ⁺ (mg · L ⁻¹)	26	1.75	93
Ca ²⁺ (mg · L ⁻¹)	< 0.1	1.8	-
Mg ²⁺ (mg · L ⁻¹)	< 0.1	0.37	-
NH ₄ ⁺ (mg · L ⁻¹)	7	0.2	97
Cl ⁻ (mg · L ⁻¹)	851	56.1	93
SO ₄ ²⁻ (mg · L ⁻¹)	2 350	< 30	99
PO ₄ ³⁻ (mg · L ⁻¹)	805	58.8	93
TKN (mg · L ⁻¹)	81	99	-
TP (mg · L ⁻¹)	723	143	-
pH	1.5	1.07	-
Conductivity (mS · cm ⁻¹)	16.8	0.74	-
Extinction (420 nm)	0.04	0.004	-

7.3 Conclusions

The complete downstream process for the production of lactic acid after fermentation of glucose from hydrolysate of shredded barley was investigated in this work. By using the steps of down streaming ultrafiltration, softening, electro dialysis, ion exchange and evaporation, a concentrated lactic acid with only a small portion of impurities is produced. Ultrafiltration and softening of the sodium lactate solution are required in order to operate the electro dialysis properly. All suspended solids are removed by ultrafiltration, and the concentration of multivalent cations is reduced to less than $1 \text{ mg} \cdot \text{L}^{-1}$ by softening. Sodium lactate was purified and concentrated by mono-polar electro dialysis up to $236 \text{ g} \cdot \text{L}^{-1}$. Subsequent purification with bipolar electro dialysis yielded good performance parameters with water transport rates of 0.17 L/eq (three-compartment electro dialysis). As a result free lactic acid concentration reached $183 \text{ g} \cdot \text{L}^{-1}$ and chemical impurities such as inorganic cations and compounds of nitrogen were considerably reduced. Additional de-colorisation and de-ionization process steps using ion exchange resins were integrated to polish the free lactic acid for high-grade applications in industry (biodegradable plastics, basic chemicals for further synthesis, biofuels) and agriculture (acidification, cleaning and preservation agents, disinfectants). This reduced the concentrations of the remaining ingredients by 93 to 99 %.

References

- ANTONIO G.V., PINELLI D., ROSSI M., FAJNER D., MAGELLI, F. MATTEUZZI D. (1996):** Production of L(+) and D(-) lactic acid isomers by *Lactobacillus casei* subsp. *casei* DSM 20011 and *Lactobacillus coryniformis* subsp. *torquens* DSM 20004 in continuous fermentation. *Journal of Fermentation and Bioengineering* 81 (6) 548-552
- ANURADHA R., SURESH A. K., VENKATESH K.V. (1999):** Simultaneous saccharification and fermentation of starch to lactic acid. *Process Biochemistry* 35 (3-4) 367-375
- ANDERSEN M., KIEL P. (2000):** Integrated utilisation of green biomass in the green biorefinery. *Industrial Crops and Products* 11 (2-3) 129-137
- BAI D.-M., JIA M.-Z., ZHAO X.-M., BAN R., SHEN F., LI X.-G., XU S.-M. (2003):** L(+)-lactic acid production by pellet-form *Rhizopus oryzae* R1021 in a stirred tank fermentor. *Chemical Engineering Science* 58 (3-6) 785-791
- BAILEY J.E., OLLIS D.F. (1986):** *Biochemical Engineering Fundamentals*. Second Edition. Mc Graw Hill, Inc.
- BAILLY M., BALMANN H., ROUX-DE A. P., LUTIN F., CHERYAN M. (2001):** Production processes of fermented organic acids targeted around membrane operations: design of the concentration step by conventional electrodialysis. *Journal of Membrane Science* 191:129-142.
- BAILLY M. (2002):** Production of organic acids by bipolar electrodialysis: realizations and perspectives. *Desalination* 144 (1-3) 157-162
- BERRY A. R., FRANCO C.M.M., ZHANG W., MIDDELBERG A.P.J. (1999):** Growth and lactic acid production in batch culture of *Lactobacillus rhamnosus* in a defined medium. *Biotechnology Letters* 21 (2) 163-167
- BOUCHOUX A., BALMAN H.R.-DE, LUTIN F. (2005):** Nanofiltration of glucose and sodium lactate solutions: Variations of retention between single- and mixed-solute solutions. *Journal of Membrane Science* 258 (1-2) 123-132
- BULUT S., ELIBOL M., OZER D. (2004):** Effect of different carbon sources on L(+)-lactic acid production by *Rhizopus oryzae*. *Biochemical Engineering Journal* 21 (1) 33-37
- BUSTOS G., MOLDES A.B., CRUZ J.M., DOMINGUEZ J.M. (2004):** Formulation of low-cost fermentative media for lactic acid production with *Lactobacillus rhamnosus* using vinification lees as nutrients. *Journal of Agricultural and Food Chemistry* 52 (4) 801-808
- CAO X., YUN H.S., KOO Y.M. (2002):** Recovery of L(+)-lactic acid by anion exchange resin Amberlite IRA-400. *Biochemical Engineering Journal* 11 (2-3) 189-196
- DANNER H., MADZINGAIDZO L., THOMASSER C., NEUREITER M., BRAUN R. (2002):** Thermophilic production of lactic acid using integrated membrane bioreactor systems coupled with monopolar electrodialysis. *Applied Microbiology and Biotechnology* 59 (2-3) 160-169
- DATTA R., TSAI S.P., BONSIGNORE P., MOON S.H., FRANK J.R. (1995):** Technological and economic potential of poly(lactic acid) and lactic acid derivatives, *FEMS Microbiology Reviews* 16, 221-231
- DUDA A., PENCZEK S. (2003):** Polylactide [poly(lactic acid)]: synthesis, properties and applications. *Polimery* 48, 16-27
- EUROPEAN UNION (2005):** Directorate-General for Agriculture and Rural Development: Agriculture in the European Union – Statistical and Economic Information 2004, February 2005
- FRIELING P. VON, SCHÜGERL K. (1999):** Recovery of lactic acid from aqueous model solutions and fermentation broths. *Process Biochemistry* 34 (6-7) 685-696

- FU W., MATHEWS A.P. (1999):** Lactic acid production from lactose by *Lactobacillus plantarum*: kinetic model and effects of pH, substrate, and oxygen. *Biochemical Engineering Journal* 3 (3) 163-170
- FUKUSHIMA K., SOGO K., MIURA S., KIMURA Y. (2004):** Production of D-lactic acid by bacterial fermentation of rice starch. *Macromolecular Bioscience* 4 (11) 1021-1027
- GLASSNER D. A., DATTA R. (1990):** Process for production and purification of lactic acid. EP 0393818 A1.
- GROHMANN K., BOTHAST R.J. (1997):** Saccharification of corn fibre by combined treatment with dilute sulphuric acid and enzymes. *Process Biochemistry* 32 (5) 405-415
- HOFVENDAHL K., HAHN-HÄGERDAHL B. (1997):** L-lactic acid production from whole wheat flour hydrolysate using strains of *Lactobacilli* and *Lactococci*. *Enzyme and Microbial Technology* 20 (4) 301-307
- HUANG H.J., YANG S.T., RAMEY D.E. (2004):** A hollow-fiber membrane extraction process for recovery and separation of lactic acid from aqueous solution. *Applied Biochemistry and Biotechnology* 113-16, 671-688
- KAMM B., KAMM M., RICHTER K. (1997):** Formation of Aminium Lactates in Lactic Acid Fermentation. Preparation and Characterization of 1,4-piperazinium-(L,L)-dilactate obtained from L(+)-Lactic Acid (Part I). *Acta Biotechnologica* 17 (1) 3-18
- KAMM B., KAMM M., RICHTER K., REIMANN W., SIEBERT A. (2000):** Formation of Aminium Lactates in Lactic Acid Fermentation. Fermentative Production of 1,4-Piperazinium-(L,L)-dilactate and its Use as a Starting Material for the Synthesis of Dilactide (Part 2). *Acta Biotechnologica* 20 (3-4) 289-304
- KIM Y. H., MOON S-H. (2001):** Lactic acid recovery from fermentation broth using one-stage electro dialysis. *J. Chem. Technol. Biotechnol.* 76:169-178
- KWON S., YOO I.K., LEE W.G., CHANG H.N., CHANG Y.K. (2001):** High-rate continuous production of lactic acid by *Lactobacillus rhamnosus* in a two-stage membrane cell-recycle bioreactor. *Biotechnology and Bioengineering* 73 (1) 25-34
- LEE E. G., MOON S. H., CHANG Y. K., YOO IK-K., CHANG H. N. (1998):** Lactic acid recovery using two-stage electro dialysis and its modelling. *Journal of Membrane Science* 145: 53-66
- MAN J.C.-DE, ROGOSA M., SHARPE M.E. (1960):** A medium for the cultivation of lactobacilli. *J. Appl. Bacteriol.* 23:130-135.
- MARTAK J., SCHLOSSER S., SABOLOVA E., KRISTOFIKOVA L., ROSENBERG M. (2003):** Fermentation of lactic acid with *Rhizopus arrhizus* in a stirred tank reactor with a periodical bleed and feed operation. *Process Biochemistry* 38 (11) 1573-1583
- MELZUCH K., VOTRUBA J., HABOVA V., RYCHTERA M. (1997):** Lactic acid production in a cell retention continuous culture using lignocellulosic hydrolysate as a substrate. *Journal of Biotechnology* 56 (1) 25-31
- MIURA S., ARIMURA T., HOSHINO M., KOJIMA M., DWIARTI L., OKABE M. (2003):** Optimization and scale-up of L-lactic acid fermentation by mutant strain *Rhizopus* sp. MK-96-1196 in airlift bioreactors. *Journal of Bioscience and Bioengineering* 96 (1) 65-69
- MOLDES A.B., ALONSO J.L., PARAJO J.C. (1999):** Cogeneration of cellobiose and glucose from pretreated wood and bioconversion to lactic acid: A kinetic study. *Journal of Bioscience and Bioengineering* 87 (6) 787-792
- MONTGOMERY R. (2004):** Development of biobased products. *Bioresource Technology* 91 (1) 1-29
- NANCIB N., NANCIB A., BOUDJELAL A., BENSLIMANE C., BLANCHARD F., BOUDRANT J. (2001):** The effect of supplementation by different nitrogen sources on the production of lactic acid from date juice by *Lactobacillus casei* subsp. *rhamnosus*. *Bioresource Technology* 78 (2) 149-153

- PARK E.Y., ANH P.N. OKUDA N. (2004):** Bioconversion of waste office paper to L(+)-lactic acid by the filamentous fungus *Rhizopus oryzae*. *Bioresource Technology* 93 (1) 77-83
- PATEL M., OU M., INGRAM L.O., SHANMUGAM K.T. (2004):** Fermentation of sugar cane bagasse hemicellulose hydrolysate to l(+)-lactic acid by a thermotolerant acidophilic *Bacillus* sp.**. *Biotechnology Letters* 26 (11) 865-868
- PAYOT T., CHEMALY Z., FICK M. (1999):** Lactic acid production by *Bacillus coagulans*--kinetic studies and optimization of culture medium for batch and continuous fermentations. *Enzyme and Microbial Technology* 24 (3-4) 191-199
- RICHTER K., BEYER F. (1998):** Cell-reuse in submerged lactic acid batch fermentation. *Acta Biotechnologica* 18 (1) 3-16
- SIEBOLD M., RINDFLEISCH D., SCHÜGERL K., FRIELING P. VON, JOPPIEN R., RÖPER H. (1995):** Comparison of the Production of Lactic Acid by Three Different *Lactobacilli* and its Recovery by Extraction and Electrodialysis. *Process Biochemistry* 30 (1) 81-95
- SIRKA K. K., SHANBHAG P. V., KOVVALI A. S. (1999):** Membrane in a reactor: A functional perspective. *Ind. Eng. Chem. Res.* 38: 3715-3737
- TANIGUCHI M., TOKUNAGA T., HORIUCHI K., HOSHINO K., SAKAI K., TANAKA T. (2004):** Production of L-lactic acid from a mixture of xylose and glucose by co-cultivation of lactic acid bacteria. *Applied Microbiology and Biotechnology* 66 (2) 160-165
- WEE Y.J., YUN J.S., PARK D.H., RYU H.W. (2004):** Biotechnological production of L(+)-lactic acid from wood hydrolyzate by batch fermentation of *Enterococcus faecalis*. *Biotechnology Letters* 26 (1) 71-74
- WILLKE TH., VORLOP K.D. (2004):** Industrial bioconversion of renewable resources as an alternative to conventional chemistry. *Applied Microbiology and Biotechnology* 66 (2) 131-142
- YIN P., NISHINA N., KOSAKAI Y., YAHIRO K., PAKR Y., OKABE M. (1997):** Enhanced production of L(+)-lactic acid from corn starch in a culture of *Rhizopus oryzae* using an air-lift bioreactor. *Journal of Fermentation and Bioengineering* 84 (3) 249-253

List of Figures

Figure 1: Sustainable Biorefinery Products Cycle (without foods).....	7
Figure 2: Decrease of pH during discontinuous fermentation.....	11
Figure 3: Time course of lactic acid formation in discontinuous cultivation of the strain <i>Lactobacillus paracasei</i> 168 on model media containing glucose in quantities of $40 \text{ g} \cdot \text{L}^{-1}$ and $100 \text{ g} \cdot \text{L}^{-1}$	12
Figure 4: Time course of lactic acid formation in discontinuous cultivation of the strain <i>Lactobacillus paracasei</i> 169 on model media containing glucose in quantities of $40 \text{ g} \cdot \text{L}^{-1}$ and $100 \text{ g} \cdot \text{L}^{-1}$	12
Figure 5: Time course of lactic acid formation in discontinuous cultivation of the strain <i>Lactobacillus paracasei</i> 172 on model media containing glucose in quantities of $40 \text{ g} \cdot \text{L}^{-1}$ and $100 \text{ g} \cdot \text{L}^{-1}$	13
Figure 6: Three-dimensional diagram of experiments.....	14
Figure 7: BIOSTAT MD (B. Braun Biotech International GmbH, Germany) equipped with a digital control unit DCU.....	15
Figure 8: Time function of lactic acid concentration in a batch cultivation of <i>L. paracasei</i> 168 (Initial glucose concentration $110 \text{ g} \cdot \text{L}^{-1}$; Temp: 28°C , pH: 5.5).....	17
Figure 9: Time function of glucose concentration in a batch cultivation of <i>L. paracasei</i> 168 (Initial glucose concentration $110 \text{ g} \cdot \text{L}^{-1}$; Temp: 28°C , pH: 5.5).....	17
Figure 10: Time function of biomass concentration in a batch cultivation of <i>L. paracasei</i> 168 (Initial glucose concentration $110 \text{ g} \cdot \text{L}^{-1}$; Temp: 28°C , pH: 5.5).....	18
Figure 11: Time functions of lactic acid and biomass productivity as well as coulometric glucose degradation rate in a batch cultivation of <i>L. paracasei</i> 168 (Initial glucose concentration $110 \text{ g} \cdot \text{L}^{-1}$; Temp: 28°C , pH: 5.5).....	19
Figure 12: Courses of the time functions of the lactic acid productivity, the specific lactate formation rate and the biomass concentration during a typical batch fermentation of the strain <i>L. paracasei</i> 168	20
Figure 13: Spherical diagram of the function $\Delta t = f(T, \text{pH})$: side view.....	23
Figure 14: Spherical diagram of the function $P_{\text{final}} = f(T, \text{pH})$	24
Figure 15: Spherical diagram of the function $Y_P = f(T, \text{pH})$	25
Figure 16: Spherical diagram of the function $dP/dt = f(T, \text{pH})$	26
Figure 17: Spherical diagram of the function $dX/dt = f(T, \text{pH})$	27
Figure 18: Spherical diagram of the function $\mu_{\text{max}} = f(T, \text{pH})$	28
Figure 19: Spherical diagram of the function $\pi_{\text{max}} = f(T, \text{pH})$	29
Figure 20: Spherical diagram of the function $\sigma_{\text{max}} = f(T, \text{pH})$	30
Figure 21: Top view optimisation diagrams of the process performance characteristics when the strain <i>L. paracasei</i> 168 is cultivated on MRS medium.....	31
Figure 22: Top view optimisation diagrams of the specific performance characteristics of the strain <i>L. paracasei</i> 168 in batch cultivation	34
Figure 23: Substance balance of grain milling and sieving	37
Figure 24: Experimental design in starch liquefying	38

Figure 25: Dendence of the degree of starch liquefaction in barley coarse meal hydrolysis with Termamyl 120 L on temperature and pH value	40
Figure 26: Dependence of the starch content in the residual solid after starch liquefaction of barley coarse meal with Termamyl 120 L on temperature and pH value	40
Figure 27: Experimental design in starch saccharification	41
Figure 28: Dependency of the degree of starch residue saccharification (DSS) on temperature and pH value	43
Figure 29: Glucose concentration after 2 hours of saccharification process	44
Figure 30: Glucose concentration after 4 hours of saccharification process	44
Figure 31: Pressing of fresh green biomass (lucerne grass)	47
Figure 32: Substance balance of lucerne pressing	48
Figure 33: Nitrogen enrichment during the nanofiltration of green juice	52
Figure 34: Lactic acid production obtained in discontinuous cultivation of <i>Lactobacillus paracasei</i> 168 on a complete MRS medium and a barley hydrolysate MRS medium at a temperature of 40.5°C and a pH value of 6.0	53
Figure 35: Productivity obtained in discontinuous cultivation of <i>Lactobacillus paracasei</i> 168 on a complete MRS medium and a barley hydrolysate MRS medium at a temperature of 40.5°C and a pH value of 6.0	54
Figure 36: Product formation of the strain No. 168 on different nutrient media.....	55
Figure 37: Yield and final concentration of lactic acid under different conditions.....	56
Figure 38: Time course of lactic acid with different nitrogen sources	57
Figure 39: Fitting of the calculated lactic acid productivity	57
Figure 40: Time course of lactic acid fermentation on barley hydrolysate.....	58
Figure 41: Typical time course of a lactic acid fermentation in chemostat mode without cell recycle	59
Figure 42: Set-up of a continuous lactic acid fermentation with cell retention by a hollow fibre module.....	60
Figure 43: Typical time course of a lactic acid fermentation with cell retention by a hollow fibre module.....	60
Figure 44: Test equipment for membrane filtration.....	62
Figure 45: Apparatus for electro dialysis experiments	64
Figure 46: The process scheme of mono-polar electro dialysis.....	65
Figure 47: The process scheme of the three- and two-compartment bipolar electro dialysis EDB	66
Figure 48: Conductivity and pH-value plotted as a function of throughput volumes measured in units of bed volumes using chelating resin RCH 46.....	69
Figure 49: Evolution of conductivity in the salt/acid and base circuit as well as a power vs. time during the two-compartment bipolar electro dialysis of solution of sodium lactate	71
Figure 50: Evolution of conductivity in the salt, acid and base circuit as well as of power vs. time during three-compartment bipolar electro dialysis of solution of sodium lactate	72

Figure 51: Conductivity and pH-value plotted as a function of throughput volumes measured in units of bed volumes by the up-flow desalting step for the anion and cation exchange73

List of Tables

Table 1:	Results of the strain screening by measuring the decrease of pH in standard medium MRS.....	11
Table 2:	Duration of batch cultivation observed with 3 selected strains on a medium with an initial glucose content of $100 \text{ g} \cdot \text{L}^{-1}$	13
Table 3:	Experimental scheme of phenotypic strain optimisation.....	14
Table 4:	Concentrations of reactants in batch fermentation with the strain <i>L. paracasei</i> 168 (initial glucose concentration: $120 \text{ g} \cdot \text{L}^{-1}$, T: 28°C ; pH: 5.5)	16
Table 5:	Measuring data and characteristic performance data (<i>Lactobacillus paracasei</i> 168).....	21
Table 6:	Measuring data and kinetic data obtained by means of model approximation.....	22
Table 7:	Results of parameter optimisation with the strain <i>L. paracasei</i> 168.....	32
Table 8:	Some possible parameter combinations for cultivating the strain <i>L. paracasei</i> 168 in batch fermentation	33
Table 9:	Results of sieve analysis.....	36
Table 10:	Results obtained in barley starch liquefaction.....	39
Table 11:	Results obtained in barley starch saccharification	42
Table 12:	Results of optimization of the conditions for the maximum glucose concentration after saccharification	45
Table 13:	Summary of the results	46
Table 14:	Results obtained in pressing freshly harvested lucerne green mass.....	49
Table 15:	Nitrogen contents of lucerne green juice and press cakes.....	49
Table 16:	Carbohydrate contents of lucerne green juice and press cakes	50
Table 17:	Contents of anorganic salts and organic acids in lucerne green juice and lucerne press cake	50
Table 18:	Comparison of the model parameter of lactic acid production	53
Table 19:	Components of sodium lactate solution after fermentation	67
Table 20:	Composition of initial and treated fermentation broth by ultrafiltration	68
Table 21:	Composition of initial and treated sodium lactate solution using chelating resin RCH 46.....	68
Table 22:	Parameter during two-level conventional electro dialysis of sodium lactate solution	70
Table 23:	Concentrations of selected ingredients in the diluate and concentrate solution after two-level conventional electro dialysis of sodium lactate solution	70
Table 24:	Concentrations of selected ingredients in the sodium lactate solution and in the lactic acid solution after two-compartment and three-compartment bipolar electro dialysis (EDB).....	71
Table 25:	Concentrations of selected ingredients in the lactic acid solution before and after desalting by cation and anion exchange resin as well as concentration by evaporation.....	73

**In der Reihe
Bornimer Agrartechnische Berichte**

sind bisher erschienen:

Heft 1	Technik und Verfahren der Landschaftspflege	1992
Heft 2	Beiträge zur Lagerung und Verarbeitung pflanzenbaulicher Produkte	1993
Heft 3	Technik und Verfahren in der Tierhaltung	1993
Heft 4	Technik und Verfahren der Landschaftspflege und für die Verwendung der anfallenden Materialien	1994
Heft 5	Verfahrenstechnik der Aufbereitung, Lagerung und Qualitätserhaltung pflanzlicher Produkte	1994
Heft 6	Biokonversion nachwachsender Rohstoffe und Verfahren für Reststoffbehandlung	1994
Heft 7	Preußische Versuchs- und Forschungsanstalt für Landarbeit und Schlepperprüffeld in Bornim 1927 bis 1945	1995
Heft 8	Qualitätssicherung und Direktvermarktung	1996
Heft 9	Konservierende Bodenbearbeitung auf Sandböden	1996
Heft 10	Anwendung wärme- und strömungstechnischer Grundlagen in der Landwirtschaft	1996
Heft 11	Computer-Bildanalyse in der Landwirtschaft Workshop 1996	1996
Heft 12	Aufbereitung und Verwertung organischer Reststoffe im ländlichen Raum	1996
Heft 13	Wege zur Verbesserung der Kartoffelqualität durch Verminderung der mechanischen Beanspruchung	1997
Heft 14	Computer-Bildanalyse in der Landwirtschaft Workshop 1997	1997
Heft 15	Technische und ökonomische Aspekte der Nutztierhaltung in großen Beständen	1997
Heft 16	11. Arbeitswissenschaftliches Seminar	1997
Heft 17	Nachwachsende Rohstoffe im Land Brandenburg Stand Aktivitäten und Perspektiven einer zukunftsfähigen und umweltgerechten Entwicklung	1998
Heft 18	Qualität von Agrarprodukten	1998
Heft 19	Computer-Bildanalyse in der Landwirtschaft Workshop 1998	1998
Heft 20	Beiträge zur teilflächenspezifischen Bewirtschaftung	1998
Heft 21	Landnutzung im Spiegel der Technikbewertung – Methoden Indikatoren, Fallbeispiele	1998

Heft 22	Kriterien der Nachhaltigkeit in der Verfahrensentwicklung für die Nutztierhaltung	1999
Heft 23	Situation und Trends in der Landtechnik / Erneuerbare Energien in der Landwirtschaft	1999
Heft 24	Institut für Landtechnik der Deutschen Akademie der Landwirtschaftswissenschaften zu Berlin 1951 bis 1965	1999
Heft 25	Computer-Bildanalyse in der Landwirtschaft Workshop 1999 / 2000	2000
Heft 26	Computer-Bildanalyse in der Landwirtschaft Workshop 2001	2001
Heft 27	Approaching Agricultural technology and Economic Development of Central and Eastern Europe	2001
Heft 28	6 th International Symposium on Fruit, Nut, and Vegetable Production Engineering	2001
Heft 29	Measurement Systems for Animal Data and their Importance for Herd Management on Dairy Cow Farms	2002
Heft 30	Produktion, Verarbeitung und Anwendung von Naturfasern	2002
Heft 31	Computer-Bildanalyse in der Landwirtschaft Workshop 2002	2002
Heft 32	Biogas und Energielandwirtschaft - Potenzial, Nutzung, Grünes Gas TM , Ökologie und Ökonomie	2003
Heft 33	Sozioökonomische Aspekte zu Perspektiven des Offenlandmanagements	2003
Heft 34	Computer-Bildanalyse in der Landwirtschaft Workshop 2003	2003
Heft 35	Energieholzproduktion in der Landwirtschaft Potenzial, Anbau, Technologie, Ökologie und Ökonomie	2004
Heft 36	High-Tech Innovationen für Verfahrensketten der Agrarproduktion. Statusseminar 2003	2004
Heft 37	Computer-Bildanalyse in der Landwirtschaft Workshop 2004	2004
Heft 38	Die Landmaschinenprüfung in der DDR 1951-1991 und ihre Vorgeschichte	2004
Heft 39	Energieverlust und Schimmelpilzentwicklung bei der Lagerung von Feldholz-Hackgut	2005
Heft 40	Computer-Bildanalyse in der Landwirtschaft Workshop 2005	2005
Heft 41	Demonstration der Langzeitwirkung bedarfsorientierter Fungizidbehandlung mit dem CROP-Meter	2005

Heft 42 Biochemicals and Energy from Sustainable Utilization of
herbaceous Biomass (BESUB)

2005

Interessenten wenden sich an:

Leibniz-Institut für Agrartechnik Potsdam-Bornim e.V.
Max-Eyth-Allee 100
14469 Potsdam

Tel.: (0331) 5699-820
Fax.: (0331) 5699-849
E-Mail: atb@atb-potsdam.de

Schutzgebühr: 13,- €