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TECHNOLOGICAL IMPROVEMENTS OF A BIOREFINERY

PhD thesis

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1 INTRODUCTION

Nowadays one of the most pressing problems is to substitute the conventional, mostly fossil material and energy sources by easily and cheaply available, industrially, agriculturally, environmentally and economically beneficial alternatives. In the 1970's the oil crisis highlighted the importance of the renewable resources and biomass-based technologies, then the agricultural overproduction and the pledge of European Union to reduce CO_2 emission gave new impetus to the biotechnological initiatives. In addition non-food purpose use of agricultural raw materials in the spirit of sustainable development has worldwide launched a new line, with a goal to establish new plants, with renewable raw materials, widely used products, using less fossil energy and producing minimal co-products and wastes. The above idea ended in the born of *biorefinery* concept, which gives alternative to the petrol-based refineries, by combining biomass-based raw materials and environmental friendly technologies and forming a complex, economic unit.

Lactic acid, produced mainly by fermentation, is needed both in food and polymer market, and due to its use as a raw material of the latter industry, lactic acid fermentation has the potential to develop to a million-tones industry in the next decades. Lactic acid is a platform chemical, intermediate of several products which were previously produced using petroleum-based technologies. One of them is its biodegradable polymer, the poly lactic acid (PLA), which is (due to the recent development of its physical properties) increasingly competitive with the petrochemical-derived plastics.

During my research work I had the opportunity to participate in several projects which had the aim to design white chemical units (biorefineries) based on renewable raw materials (wheat and sweet sorghum). The future main product of these plants is lactic acid and its derivatives, accordingly my research primarily pointed at the technological development of lactic acid fermentation. My global target was finding a technological solution applicable to both raw materials and capable of cost-effective production at the same time, and integrating further producing units based on lactic acid so that the resulting complex can become a third phase biorefinery, which is able to flexibly adapt to market demand and available raw materials.

2 BACKGROUND

Biorefineries combine the necessary technologies between biological raw materials and industrial intermediates and final products[1]. The raw materials, the technologies and the main products should be chosen so that the plant can be flexibly changed according to the conditions and needs.
A possible main product of the biorefinery is the versatily used platform chemical, namely *lactic acid*. The industrial production of lactic acid goes back to several decades, and although synthetic production was also established in the 1960's, fermentative production currently has a greater significance, because of the renewable raw materials and the optically pure final product (L(+)- or D(-)-lactic acid). Mainly its use in food industry has great importance, but since 2002 there was a large jump in its use in polymer production as well. Poly lactic acid (PLA) is derived from the dimer of lactic acid by ring opening polymerization, and it is a diversely used, biodegradable plastic. Due to its biodegradability, thermoplastic properties and other favorable physical parameters it becomes more and more realistic alternative in the plastic market[2].

Literature of lactic acid fermentations is abundant, and numerous publications, industrial patents and patented strains were released in the last 60 years. From industrial point of view application of homofermentative strains is practical, which convert carbon sources to lactic acid (and energy for maintenance) without other final product (from 1 mol sugar to 2 mol lactic acid and 2 mol ATP).
Although use of bacteria is preferred in most cases, *filamentous fungi* such as *Rhizopus*, *Mucor*, *Monilia* strains are able to produce lactic acid as well, by aerobic metabolism. The disadvantage of this lactic acid production is the special yeast cell metabolism of fungi (at given circumstances they act as yeast cells, producing carbon dioxide and ethanol), beside they incorporation some part of the raw material into their biomass during cell growth, decreasing lactic acid yield.
Lactic acid bacteria (LAB) belong to *Lactobacillus*, *Streptococcus*, *Leuconostoc* and *Enterococcus* genera. Most of them are protected by patents, and the majority is the strains of *Lactobacillus* genus. Much of LAB can use only simple sugars (mono- and disaccharides), accordingly physical and/or chemical pre-treatment and enzymatic hydrolysis of starchy, cellulosic or lignocellulosic substances is indispensable. One of the most common techniques is separate hydrolysis and fermentation (SHF – hydrolysis precedes fermentation), but simultaneous saccharification and fermentation (SSF – the two process run in line) is also implemented in practice. *Amyloytic* bacteria and filamentous fungi can use starch directly (direct fermentation), because they own liquefying and saccharifying enzymes. The disadvantage of lactobacilli is their mesophilic property (they grow below 45°C), which limits their industrial use because of the high risk of contamination at this temperature.
Thermophilic lactic acid production has been concerned by both researchers and industry for decades. The use of thermophilic or thermotolerant bacteria (and fungi) can reduce the risk of contamination, and furthermore theoretically higher conversion and productivity can be reached by these strains. On the other hand SSF under thermophilic conditions is easier, because the temperature optimum of saccharification and fermentation overlaps. The most widely used strains belong to the thermotolerant *B. coagulans* species, and good results have been achieved by the thermophilic *Bacillus stearothermophilus* or *Geobacillus stearothermophilus* strains as well[3].

The ideal *raw material* of biotechnological lactic acid fermentation is cheap, containing minimal contamination, easily produced, cultivated with high yield, resulting minimal co-products and

[1] B. Kamm, M. Kamm (2004) Principles of biorefineries, *Applied Microbiology and Biotechnology* **64**: 137-145
[2] Y.-J. Wee, J.-N. Kim, H.-W. Ryu (2006) Biotechnological Production of Lactic Acid and Its Recent Applications, *Food Technology and Biotechnology* **44**: 163-172
[3] H. Danner, R. Braun (1999) Biotechnology for the production of commodity chemicals from biomass, *Chemical Society Reviews* **28**: 395-405

wastes, needing minimal pre-treatments and available throughout the year. Possible carbon sources can be sugar (such as sugar beet, sugar cane, sweet sorghum, whey), starch (such as wheat, corn, potato, rice) or cellulose/lignocellulose containing raw materials. LAB use carbon source to lactic acid and energy production, but they have a complex nutrient need, because they are not able to synthesize some growth factors (B vitamins, amino acids). For their growth they need nitrogen sources in form of amino acids and peptides (organic nitrogen), and supplementation of vitamins and minerals is indispensable, too. Consequently for industrial lactic acid production besides appropriate, easily usable carbon source, choose of the proper supplementation and medium optimization is crucial.

Wheat is an appropriate first-generation fermentation raw material due to its high starch content. Its use for lactic acid fermentation is not a novelty: according to literature[4] after enzymatic treatment starch content as carbon source, and protein content as nitrogen source can be used, however additional supplementation (yeast extract) is needed in most of the cases for an effective lactic acid production. The high sugar containing *sweet sorghum* is a potential raw material as well. The pressed or extracted, glucose, fructose and saccharose containing sweet sorghum juice has versatile use: ethanol, methanol, amino acid and lactic acid[5] fermentation researches are based on this raw material. Besides high sugar content the juice has minimal protein level, therefore supplementation of sweet sorghum based medium is needed. In Hungary industrial use of this plant is complicated by the fact that it can be harvested once a year causing problems in juice pressing and storage. Namely during harvest and pressing wild yeast and bacterium strains get into the juice from the surface of the straw, causing drastic decrease of sugar content and deterioration of the juice. Long-time storage of the plant is not solved, in smaller scale evaporation, chemical or heat-treatment of the juice, in larger scale silage of the plant can be the solution.

Temperature and *pH* are also important factors in lactic acid fermentation, consequently examination of these parameters also contribute to the effective, fast and industrially competitive lactic acid production. Several studies deal with the effect of pH and temperature, integrating it into the fermentation kinetic models. The first kinetic description of lactic acid production was established by Luedeking and Piret[6], and they introduced the concept of growth-associated and nongrowth-associated production of primery metabolites. In the last decades this model was supplemented by the effect of pH, temperature, oxygen-level, inhibition by substrate or undissociated lactic acid, and different medium supplementations on lactic acid production. While temperature can be easily controlled during fermentation, maintaining pH at a constant level is more complicated. Without pH-regulation its value decreases continuously in line with lactic acid production, and at a critical level it blocks microbial activities. To maintain constant pH level different methods can be applied: lactic acid can be neutralized by adding base or buffer to the medium, or lactic acid can be removed by extraction, adsorption or electrodialysis. The pH optimum of LAB is usually between 5 and 7.

The goal of my research work was technological development of the fermentation units of a biorefinery, with a main product of lactic acid, and using glucose, wheat starch or sweet sorghum juice as raw material. As a co-product of the biorefinery I chose yeast extract needed for lactic acid fermentation, and theoretically I dealed with the use of sweet sorghum plant for yeast fermentation.

[4] K. Hofvendahl, C. Akerberg, G. Zacchi, B. Hahn-Hagerdal (1999) Simultaneous enzymatic wheat starch saccharification and fermentation to lactic acid by *Lactococcus lactis*, *Applied Microbiology and Biotechnology* **52**: 163-169

[5] Richter K., Berthold C. (1998) Biotechnological Conversion of Sugar and Starchy Crops into Lactic Acid, *Journal of Agricultural Engineering Research* **71**: 181-191

[6] R. Luedeking, E. L. Piret (1959) A kinetic study of the lactic acid fermentation. Batch process at controlled pH, *Journal of Biochemical and Microbiological Technology and Engineering* **1**: 393-412

Lactic acid fermentation was approached via two routes (*Figure 1*): the main course was the development of a mesophilic technology, then I examined the possibility of thermophilic lactic acid production. In frame of mesophilic technology development I optimized media based on the different raw materials, investigated the nutrient requirements of the bacterium, the technological parameters of the fermentation and the scale-up of the whole process. To develop thermophilic lactic acid fermentation technology I performed two multi-step screenings, and I attempted to adapt the chosen strain to the wheat and sweet sorghum based media. Finally I examined possible ways to produce yeast extract as the key supplement of lactic acid fermentation.

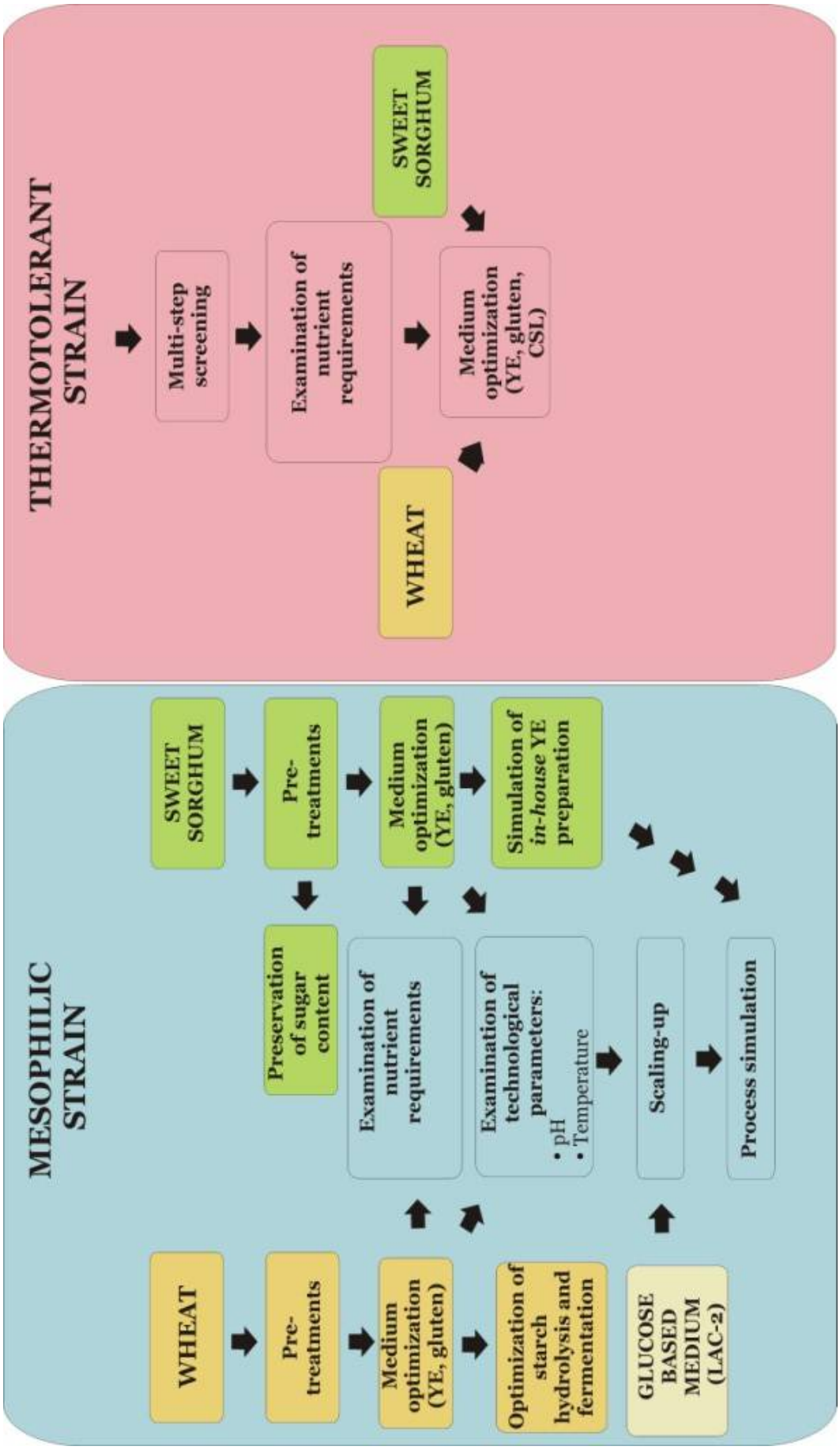

Figure 1: Overview of the research theme

3 EXPERIMENTAL METHODS

Strains

In mesophilic lactic acid fermentation experiments the following strains were used: *Lactobacillus* sp. TUB B-207 and *Lb*. sp. MKT-878 (NCAIM B02375). Thermophilic fermentations were performed with the following 21 strains: *Bacillus coagulans* (DSM 1, 2308, 2311, 2312, 2314, 2350, 2356, 2383, 2384, 2385), *B. smithii* (DSM 2319), *B. stearothermophilus* (DSM 2334, 2349), *Geobacillus stearothermophilus* (DSM 22^T, 297, 456, 494, 2027, 2313, 3299, 5934).

Media

According to the recommendation of the culture collections to revive and store *Lactobacillus* strains MRS (De Man, Rogosa és Sharpe[7]) medium, while in case of *Bacillus*, *Geobacillus* strains nutrient medium were used.
The *glucose* based experiments were performed on Lac-2 medium, with yeast extract and corn steep liquor as nitrogen sources. For *wheat* based fermentation medium wheat flour type 550 (all-purpose commercial flour) was the raw material. In some experiments gluten fraction of the wheat was separated from starch content, other experiments were done by using wheat proteins as nitrogen source after enzymatic hydrolysis of gluten. For *sweet sorghum* based experiments four types of sweet sorghum juice were available: Monori édes, Sucrosorgho, Autan and Urja.
For enzymatic treatments (wheat fractionation, hydrolysis of starch and proteins) the following enzyme products were applied according to the manufacturer's (Novozyme) recommendation: Shearzyme® 500 L (xylanase), Termamyl® SC (α-amilase), SAN®Super 240 L (gluco-amilase and protease), Alcalase® 2.4 L FG (protease), Neutrase® 0.8L (protease).
During medium optimization different carbon sources (wheat starch, sweet sorghum juice, glucose, saccharose, xylose) were supplemented with nitrogen containing components (corn steep liquor, yeast extract, gluten) and with microcomponents (vitamins, salts, amino acids).
Sterilization of media was performed at 120°C, for 20 minutes, except vitamins and amino acids that were sterile filtrated. Carbon sources were treated separately from other components.

Fermentation

For media optimization shaking flask experiments were performed in 100/200 mL scale. For larger scale I used the following fermentors: B. Braun Biostat® Q (500-800 mL), B. Braun Biostat® M (1000-1800 mL), B. Braun Biostat® U (15-20 L), B. Braun Biostat® 300D (150-230 L). For pH regulation $CaCO_3$, 20% NH_4OH, 20% NaOH, 20% trimethyl amine, 20% dimethyl amine and 25% H_2SO_4 were used.

Analyses

Substrates and products were analyzed with Waters Breeze HPLC System. Method for acid containing samples: 5 mM H_2SO_4 mobile phase (0.5 mL/min), BioRad Aminex HPX-87H column, 65 °C, detector temperature of 40°C. Method for sugar containing samples: Millipore water mobile phase (0.6 mL/min), BioRad Aminex HPX-87P column, 85°C, detector temperature of 40°C.
To follow cell growth optical density was measured photometrically (A_{600}).

[7] De Man, Rogosa, Sharpe (1960) Medium for the cultivation of lactobacilli, *Journal of Applied Bacteriology* **23:** 130-135

4 RESULTS

The aims of my PhD thesis is the technological improvements of a biorefinery's fermentation units, on different raw materials, with various strains, under mesophilic and thermophilic conditions, and this topic also included the issues of raw materials. The following summarizes the results of the specific topics.

Glucose based experiments on Lac-2 medium

Two mesophilic lactic acid producers (*Lactobacillus* sp. TUB B-207 and *Lactobacillus* sp. MKT-878) were tested on a previously optimized lactic acid fermentation medium named Lac-2. Showing respectful lactic acid yield and productivity results (0.88 g/g, 3.52 g/L*h) MKT-878 strain proved to be more effective. With the chosen strain parallel experiments were conducted at laboratory scale (0.8 L), which demonstrated that the fermentation was reproducible with a minimal variance. On 0.8-20-200 L scales product yield improved by increasing scale, and productivity reached the industrially acceptable 3 g/L*h value in each case.

Wheat based fermentations

After fractionation of wheat flour experiments were performed on the hydrolyzed starch content of wheat. Without supplementation fermentation showed weak productivity results, accordingly I decided to supplement the medium with nitrogen containing compounds: fermentations with yeast extract, corn steep liquor and yeast autolysate each in different concentration were tested. Yeast extract supplementation proved to be the most convenient in an amount of 20 g/L (2 g/L total nitrogen content) for ~120 g/L glucose containing medium.

To partly substitute yeast extract protease-treated gluten fraction was used as nitrogen source, then realizing a 3^2 experimental design I optimized the appropriate ratio of gluten and yeast extract to maximize productivity. With the optimal composition (16 g/L gluten, 8 g/L yeast extract) fermentation resulted in 0.88 g/g yield and 3.54 g/L*h productivity, and these results were reproductable on 0,8-1,5-15 L scale as well.

Beside separated hydrolysis and fermentation (SHF) I examined other techniques as well. Since direct fermentations with *L. amylovorus* and *Rhizopus oryzae* strains have not taken satisfactory results, I turned to simultaneous saccharification and fermentation (SSF). In that technique saccharification of starch and fermentation of the resulted glucose are going in parallel, reducing the demand of fermentor capacity and eliminating the risk of substrate inhibition. The disadvantage of SSF is that the pH and temperature optimum of the two processes do not overlap, so both procedures run too slowly. To avoid this effect I applied and optimized a so-called combined hydrolysis and fermentation (CHF) technique, in which hydrolysis and fermentation take place in the same reactor, but there is a time delay in inoculation and cut down of hydrolysis time before fermentation. To find an appropriate inoculation time point, four different settings were applied (0, 12, 24, 36 hours), and an empiric kinetic model was developed. This model gave an optimal inoculation time point (14 hours) for the maximal productivity, and this setting gave good outcome experimentally as well: included the time of both hydrolysis and fermentation productivity almost reached the critic 3 g/L*h value.

Sweet sorghum based fermentations

After examined the sugar content of the four sweet sorghum juice types and the possible pre-treatments, I chose the centrifuged Monori édes juice as fermentation raw material, because this juice had the highest fermentable sugar content (saccharose, glucose, fructose). In 2009 this subspecies did not show attractive sugar yield, therefore experiments were continued on Sucrosorgho type.

For the preservation of sugar content of the juice the most convenient solutions were freezing in smaller case, and heat treatment or acidification (pH 2.0-2.5) in larger scale. To preserve sweet

sorghum chops I tested various chemical, physical and biological methods (separately or in combination): the combined treatments gave better results, but the effective preservation of sugar content is still not solved.
Since protein content of sweet sorghum juice is negligible, optimization of the medium was started by examination of nitrogen supplementation. For Monori édes juice (with ~120-130 g/L sugar content) 20 g/L yeast extract was sufficient. To reduce the amount of yeast extract another 3^2 experimental design was generated, choosing yeast extract and gluten as factors. The optimal setting (16 g/L gluten, 6 g/L yeast extract) gave 0.99 g/g product yield and 3.04 g/L*h productivity. On the basis of the higher sugar containing sweet sorghum juice (Sucrosorgho, 2009), fermentations did not reach these results, accordingly I had to examine the carbon, nitrogen and microcomponent need of the strain, and to reveal their interaction.

Examination of nutrient requirements of the strain
Based on the above results on 120 g/L carbon source level 16 g/L gluten (2 g/L total nitrogen content) and 6 or 8 g/L yeast extract seemed to be essential, and the yeast extract acted as microcomponent source. To substitute the expensive yeast extract I examined the vitamin and amino acid needs of the strain by negative tests. Six vitamins and three amino acids proved to be essential, and lack of further nine amino acids caused a slower lactic acid production. Applying these components in this composition failed to substitute yeast extract.
Investigating interaction of the carbon, nitrogen and microcomponent sources by response surface methodology I found that increasing carbon source level the nitrogen need increased linearly, while the demand of microcomponents (yeast extract) under 60 g/L carbon source level showed linearity, but over this limit it was independent of carbon source level.
Applying the above relation on higher sugar containing (~150-180 g/L) sweet sorghum juice, the medium was supplemented by 24 g/L gluten and 8 g/L yeast extract, and the fermentation gave 3.54 g/L*h productivity. This result was reproducted in laboratory scale with small variation, and the fermentation was scaled up (0.5-1.8-18 L) with stable results.

Examination of pH and temperature of fermentation
To neutralize the produced lactic acid and to maintain a constant pH during fermentation I used five different agents. Among methods with calcium carbonate, ammonium hydroxide, sodium hydroxide, trimethyl amine and dimethyl amine the regulation by ammonium hydroxide and trimethyl amine gave the best results. The disadvantage of ammonium hydroxide is the producing ammonium lactate which causes product inhibition in high sugar containing media (resulting high ammonium lactate concentration). Buffering the broth by calcium carbonate is either inconvenient because of the large amount of gypsum produced during down-stream processes, or because a new phenomenon showed up, possibly caused by the aggregation of calcium lactate: over a given calcium lactate concentration the fermentation broth became solid, preventing to complete total conversion of sugars into lactic acid. Combining the two agents these issues can be solved: adding 22 g/L calcium carbonate at the start of fermentation, then using automatic pH-regulation by ammonium hydroxide, fermentation gave 0.85 g/g yield and 3.55 g/L*h productivity on a 180 g/L glucose containing Lac-2 medium.
To find optimal pH and temperature range of the strain a 4^2 experimental design of was generated: fitting a Gauss surface onto the productivity results, 35.5°C and 7.0 pH values were found optimal. Since the pH had a broad optimum range, I continued to apply the previously set pH 5.8-6.0 value.

Thermophilic lactic acid production
Thermophilic lactic acid experiments started by a multi-step screening with 10 *Bacillus coagulans* strains. The chosen strain was the *B. coagulans* DSM 2356, which is able to grow and produce lactic acid homofermentatively at 52°C even on high glucose concentration. According to literature *Bacillus/Geobacillus stearothermophilus* strains are also suitable for thermophilic production,

therefore a new multi-step screening was established with three previous *B. coagulans,* one *B. smithii* and ten *Bacillus/Geobacillus stearothermophilus* strains. After these investigations the DSM 2356 strain proved to be the best lactic acid producer again, at 55°C and on high saccharose concentration as well.
To optimize media the nutrient need of the strain was examined at 120 and 180 g/L carbon source level, then by response surface methodology I attempted to substitute yeast extract by gluten and corn steep liquor. On the basis of these results at 120 g/L carbon source level 20 g/L, while at 180 g/L level 30 g/L yeast extract was required, and substitution of these amounts did not succeed.

Limitations
Either in case of mesophilic or thermotolerant strain limiting factors encountered, that are relevant in the planning process. These effects are summarized in *Table 1*.

Table 1.: Summary of limitations and inhibitions of the mesophilic and thermotolerant strain

	Mesophilic (*Lactobacillus* sp. MKT-878)	**Thermotolerant (*Bacillus coagulans* DSM2356)**
Limiting substrates before media optimization	▪ Nitrogen source ▪ Microcomponent source	▪ Microcomponent source ▪ Partly nitrogen source
Product inhibition	▪ <30 g/L lactic acid: lactic acid in acid form ▪ <110-120 g/L lactic acid: calcium lactate, sodium lactate ▪ >110-130 lactic acid: ammonium lactate	▪ <20 g/L lactic acid: lactic acid in acid form, calcium lactate (adaptation of cells is possible) ▪ >80-100 g/L lactic acid: ammonium lactate
Substrate inhibition	None, but higher initial sugar concentration causes weaker product yield	None, but higher initial sugar concentration causes weaker product yield

In case of mesophilic strain these factors were eliminated by the suitably chosen technique, however in case of thermotolerant strain to avoid product inhibition additional investigation is needed (for example modifying pH-regulation, removing lactic acid form the fermentation broth, diluting the media, applying fed-batch or continuous techniques).

5 THESES

1. A wheat based fermentation medium was successfully optimized, using the hydrolyzed starch content of wheat as carbon source, one part of the hydrolyzed protein fraction (gluten) as nitrogen source, and yeast extract as microcomponent source, and this optimization was resulted in the following facts: for the ~120 g/L glucose containing starch hydrolysate 16 g/L gluten (app. 2 g/L total nitrogen content) and 8 g/L yeast extract was needed for a 3.54 g/L*h productivity and a 0.88 g/g product yield result. The technique is reproducible and scaling-up was successful. (Hetényi et al., 2010d)

2. The advantages of direct fermentation, separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) were compared, and a combined (starch) hydrolysis and fermentation (CHF) technique was developed. The optimal inoculation time point was determined in 14 hours, and by this technique on the previously

optimized wheat flour based medium 2.88 g/L*h productivity resulted, included the total time of hydrolysis and fermentation processes. (Hetényi et al., 2010a)

3. A sweet sorghum juice based fermentation medium was successfully optimized, using the sugar content of the juice as carbon source, the added gluten as nitrogen source, and yeast extract as microcomponent source, and this optimization was resulted in the following facts: for the ~120 g/L inverted saccharose containing sweet sorghum juice 16 g/L gluten (app. 2 g/L total nitrogen content) and 6 g/L yeast extract was needed for a 3.04 g/L*h productivity and a 0.99 g/g product yield result. The technique is reproducible and scaling-up was successful. (Hetényi et al., 2010c)

4. Following the results of the two media optimization, nitrogen and microcomponent requirements of the used *Lactobacillus* sp. MKT-878 (NCAIM B02375) strain was determined at a specific carbon source level, and essential vitamin and amino acid needs were defined:
   - nitrogen need of the strain increases linearly with the level of carbon source, while microcomponent need increases linearly up to 60 g/L carbon source level, over this value its demand is constant. (Hetényi et al., 2010e)
   - for lactic acid fermentation 6 vitamins are essential for the used strain: biotin, nicotinic acid, pantothenic acid, pyridoxine, riboflavin and thiamine.
   - for lactic acid fermentation 3 amino acids are essential for the used strain: aspartic acid, glutamic acid and serine, while lack of additional 6 amino acids causes deceleration of lactic acid production and cell growth (arginine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, valine).
   - because of the numerous essential microcomponents the total substitution of yeast extract is uneconomical, however its amount can be reduced, if yeast extract acts only as microcomponent source beside other supplemented nitrogen source.

5. It is concluded that on a high carbon source containing (>120 g/L) medium pH-regulation by ammonium hydroxide leads to product inhibition by ammonium lactate, while buffering by calcium carbonate causes aggregation of calcium lactate and incomplete fermentation. Combining these methods (adding 22 g/L calcium carbonate at the start of fermentation) both problems have been solved. (Hetényi et al., 2010b)

6. The pH and temperature optimum of *Lactobacillus* sp. MKT-878 (NCAIM B02375) was examined, and 35,5 °C and pH 7,0 were found to be optimal. (Hetényi et al., 2010b)

7. Through a multi-scale screening research thermotolerant *Bacillus coagulans* DSM2356 lactic acid producer strain had been chosen, and the following facts were concluded:
   - the strain is able to grow on saccharose, glucose and fructose carbon sources, tolerating even up to 180 g/L initial sugar concentration.
   - the strain prefers micro-aerophil conditions for the growth.
   - applying yeast extract as nitrogen source, the sufficient amount for 120 g/L carbon source level is 20 g/L, while for 180 g/L level is 30 g/L.
   - combining yeast extract with different nitrogen sources, the effect of the other nitrogen containing supplementation is not significant on lactic acid production.
   - using ammonium hydroxide for pH-regulation ammonium lactate corresponding to 80 g/L lactic acid concentration causes product inhibition, while in case of calcium carbonate calcium lactate has not inhibiting effect.

6 CONCLUSIONS, POSSIBLE APPLICATIONS

Utilization of the fermentation process(es) developed during my theses work can be realized in two running projects.
Until the end of 2011, we have been participating in the development of an agro-industrial model in the frames of the project entitled "A cukorcirok integrált mezőgazdasági termelési, tárolási, feldolgozási és logisztikai rendszerének kidolgozása" (Development of an integrated agricultural production, storage, processing and logistic system for sweet sorghum), using the results of our researches on lactic acid fermentation and yeast extract production. The indirect aim of this work (supported by NKTH) is to create an R&D and innovative background capable of continuously supporting the production and expanding the consumption.
The long-term aim of a prospective biorefinery to be established in Balatonfűzfő (by Nitrokémia Co.) is to produce a high-quality and cost-effective platform compound (lactic acid), which can be an appropriate raw material for the production of a biodegradable plastic (PLA), thus creating an environmentally friendly, white biotechnological unit.

These conceptions gave the frame of my research activity, which intended to improve technological development of the planned biorefinery's lactic acid fermentation unit. The global aim of this work was to find a technological solution applicable to both raw materials and capable of cost-effective production at the same time. Accordingly I examined two fermentation raw materials, optimized fermentation media based on these resources, and I analyzed the technological parameters of lactic acid fermentation. My results (taken into account the final lactic acid concentrations as well as the overall productivities and sugar conversions) possibly create the technological bases of the realization of a feasible lactic acid fermentation unit of the planned biorefinery either on the bases of starch or sweet sorghum raw materials.

7 RELATED PUBLICATIONS

Written publications

Papers

K. Hetényi, Á. Németh, B. Sevella (2010a) Investigation and modeling of lactic acid fermentation on wheat starch via SSF, CHF and SHF technology, *Periodica Polytechnica* (beküldve)

K. Hetényi, Á. Németh, B. Sevella (2010b) Role of pH-regulation in lactic acid fermentation: second steps in a process improvement, *Chemical Engineering and Processing: Process Intensification*, IF (2009): 1,742, I: 0 (beküldve)

K. Hetényi, K. Gál, Á. Németh, B. Sevella (2010c) Use of sweet sorghum juice for lactic acid fermentation: preliminary steps in a process optimization, *Journal of Chemical Technology and Biotechnology* **85**: 872-877, IF (2009): 2,045, I: 0

K. Hetényi, Á. Németh, B. Sevella (2010d) First steps in the development of a wheat flour based lactic acid fermentation technology. Culture medium optimization, *Chemical and Biochemical Engineering Quarterly* **24**: 195-201, IF (2009): 0,387, I: 0

K. Hetényi, Á. Németh, B. Sevella (2008a) Examination of medium supplementation for lactic acid fermentation, *Hungarian Journal of Industrial Chemistry* **36**: 49-53

Hetényi K., Németh Á., Sevella B. (2008b) Fehér biotechnológiai kutatások, *Magyar Kémiai Folyóirat*, Kémiai Közlemények, **114**: 102-106

Patent

Hetényi K. Zs., Németh Á., Sevella B., Kovács L. P., Bodnár Zs. (2010d) Eljárás haszonnövények fermentációs felhasználására tejsav és származékainak előállítása céljából, P1000061 (patent application: january of 2010)

Oral presentations

Hetényi K., Németh Á., Sevella B.: Researches on nutritive requirements of a lactic acid bacterium in technological aspect, Conference of Chemical Engineering ’10, Hungary, Veszprém, 27–29 April 2010 (hungarian)

Hetényi Kata: Technological optimization of a biorefinery, Conference of Szent-Györgyi Albert College named A Biotechnológia a biológia, a kémia és a mérnöki tudományok, Hungary, Budapest, 20 March 2010 (hungarian)

Hetényi K.: Faculty of Chemical and Bioengineering, VIIth Conference of Oláh György Doctoral School, Hungary, Budapest, 4 February 2010 (hungarian)

K. Hetényi, Á. Németh, B. Sevella: Lactic acid fermentation on wheat flour via SHF and SSF technology, 2nd Central European Forum for Microbiology (CEFORM),Hungary, Keszthely, 7–9 October 2009 (english), abstract: Acta Microbiologica et Immunologica Hungarica 56 (2009) 167–168

K. Hetényi, Á. Németh, B. Sevella: Vitamin supplementation of lactic acid fermentation medium, Congress of Hungarian Society for Microbiology, XIth Fermentation Colloquium, Hungary, Keszthely, 15-17 October 2008, abstract: Acta Microbiologica et Immunologica Hungarica 56 (2009) 36K.

Hetényi, Á. Németh, B. Sevella: Examination of medium supplementation for lactic acid fermentation, Conference of Chemical Engineering ’08, Hungary, Veszprém, 22–24 April 2008 (hungarian)

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