Budapest University of Technology and Economics

New methods of utilizing the by-products of corn growing and processing

PhD Thesis

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1. Introduction

Many by-products are produced in huge quantities during the process of corn growing and processing. 11 million tons (more than the quantity of the seed) of corn stover, 100 thousand tons of corn fibre (9.5% of the processed corn), and about 35 thousand tons of corn germ cake (3.5% of the processed corn) is produced annually in Hungary. The corn stover is chopped and dispersed on the fields, the corn fibre and the germ cake are marketed as low value feedstocks. The utilization of these by-products is a current issue. The corn fibre and the germ cake arises at the corn processors on site, so the most convenient solution would be if the processors could utilize these fractions. Thus an important aspect of my researches was that the new method of utilizing these by-products should be adoptable by the processors.

There are basically two approaches of biorefining. One utilizes the biomass in one block (for example by pyrolysis), the other separates the fractions of the biomass. My researches are based on the latter approach. In the case of the corn stover value added products are the monosaccharides (glucose, xylose, arabinose). In the case of corn fibre and germ cake the lipids are also valuable fractions, especially the phytosterols and phytosterol-esters. Glucose can be utilized in a corn mill, for example to produce ethanol.

The more specific research exercises are the following:

1. Accurate analysis of the raw materials. Discussion of the results.
2. Sharp separation and high yield of the valuable components (mentioned above) by using physical, chemical and biochemical methods with minimal energy and reagent demand.
3. Testing the effect of the dilute sulphuric acid pretreatment at low temperature (T=120ºC), which was not tested previously.
4. Analysis of the liquid and the solid phases after each process, calculation of mass balances.
5. Verification of the applied analytical methods, correction, development of them, if needed and possible.
6. Statistical analysis of the results, discussion.

2. Literature background

Lignocelluloses consist of a complex, fibrous structure of cellulose, hemicelluloses and lignin, which is highly resistant to physical, chemical and biological effects. The lignin case surrounds the fibres, thus it ensures advanced recalcitrance.

Cellulose
The most common polymer of the biosphere. The linear cellulose molecules are set up from ß-D-anhydroglucose units, which are bound by ß-1,4 binds into the energetically optimal “chair” conformation. The degree of polymerisation in the cellulose can be very different (15-15000) depending on the origin of the cellulose. The cellulose – in spite of consisting of hydrophilic molecules – is insoluble in water. Largest quantity of cellulose is utilized by the paper and textile industries. The recycling of the waste paper spreads all around the world. Cellulose insulation made from recycled newsprint is becoming popular as an environmentally preferable material for building insulation. Cellophane and rayon are also produced from cellulose. Cellulose is also utilized as dietary fibre, since it enhances human digestion. Microcrystalline cellulose (E460i) and powdered cellulose (E460ii) are used as inactive fillers in tablets and as thickeners and stabilizers in processed foods. Cellulose is used to make hydrophilic and highly absorbent sponges, as well as water-soluble adhesives and

binders such as methyl cellulose and carboxymethyl cellulose which are used in wallpaper paste. Inorganic acids or enzymes hydrolise cellulose to glucose, which can be fermented to ethanol by commercial yeast (*Saccharomyces cerevisiae*) at the temperature of 30ºC.

Hemicelluloses
Hemicelluloses are the heteropolysaccharides of five carbon sugars (pentoses), with lower (20-300) degree of polymerisation, more side chains, which are bound to the cellulose by hydrogen bridges, and to the lignin by covalent bonds. The utilization of the hemicellulose is the scope of many current researches. The production of ethanol is rather a theoretical option. The utilization of the pentoses or its derivatives as food additives is possible. Typical derivatives are the xylitol, which is a diabetic sweetener, it can be produced by the hydrogenation of the xylose, and the trihydroxy-glutaric acid, which can be produced by the further oxidation of the xylitol. The third option is producing bio-surfactants (soaps, emulsifiers) through the addition of fatty acids onto the pentoses.

Lignin
It is a three dimensional aromatic polymer, containing phenolic hydroxyl- and methoxyl groups, consisting mainly of cynnamyl alcohol and gvajacol. The lignin can be utilized primarily by combustion (cogeneration of heat and electricity), or by pyrolysis.

Phytosterols, phytosterol-esters
The effects of these components in humans are not all well known, but the most important is definitely the decrease of blood LDL-cholesterol level, thus the prevention of cardiovascular diseases. The most casualties (53.4%) in Hungary since the middle of the XX'th century can be traced back to cardiovascular diseases. 6-8% total phytosterol concentration is detected in the corn fibre oil, while the commercial edible corn oil contains only 0.45%. The isolated phytosterols can be utilized many ways, for example by making functional foods.

Lignocellulosic bioetanol
The hydrolysis of the lignocellulosic polysaccharides can take place in two ways: with inorganic acids or using enzymes. However, the highly stable lignocellulose structure needs some kind of pretreatment in order to be digestible by enzymes. The most important types of pretreatment are: dilute acid/alkali pretreatment, steam explosion, wet oxidation, AFEX, Ammonia Recycle Percolation (ARP). In my researches I used dilute sulphuric acid pretreatment, this process solubilises primarily the hemicellulose, thus making the cellulose more accessible for the enzymes.

The production of lignocellulosic ethanol can only be economical if value added products are produced from all of the fractions of the raw material. The final goal of the utilization of lignocelluloses is to maximize the value which is yielded from the whole raw material. Thus it is important to separate and utilize the hemicellulose and the lignin besides ethanol production.

3. Materials and methods

I air dried the raw materials, then I performed the following analyses: dry matter, polysaccharide (starch, cellulose, xylan, arabinan), acetate, lignin, oil (sterols, sterol esters), protein, ash contents. The determination of the polysaccharide content was performed by an Aminex HPX-87H type, ion exclusion HPLC column, and an RI detector (type: LKB 2145).

The sterol, sterol ester contents were determined by using an HP 6890 type gas chromatograph (column: HP-1MS, detector: FID).

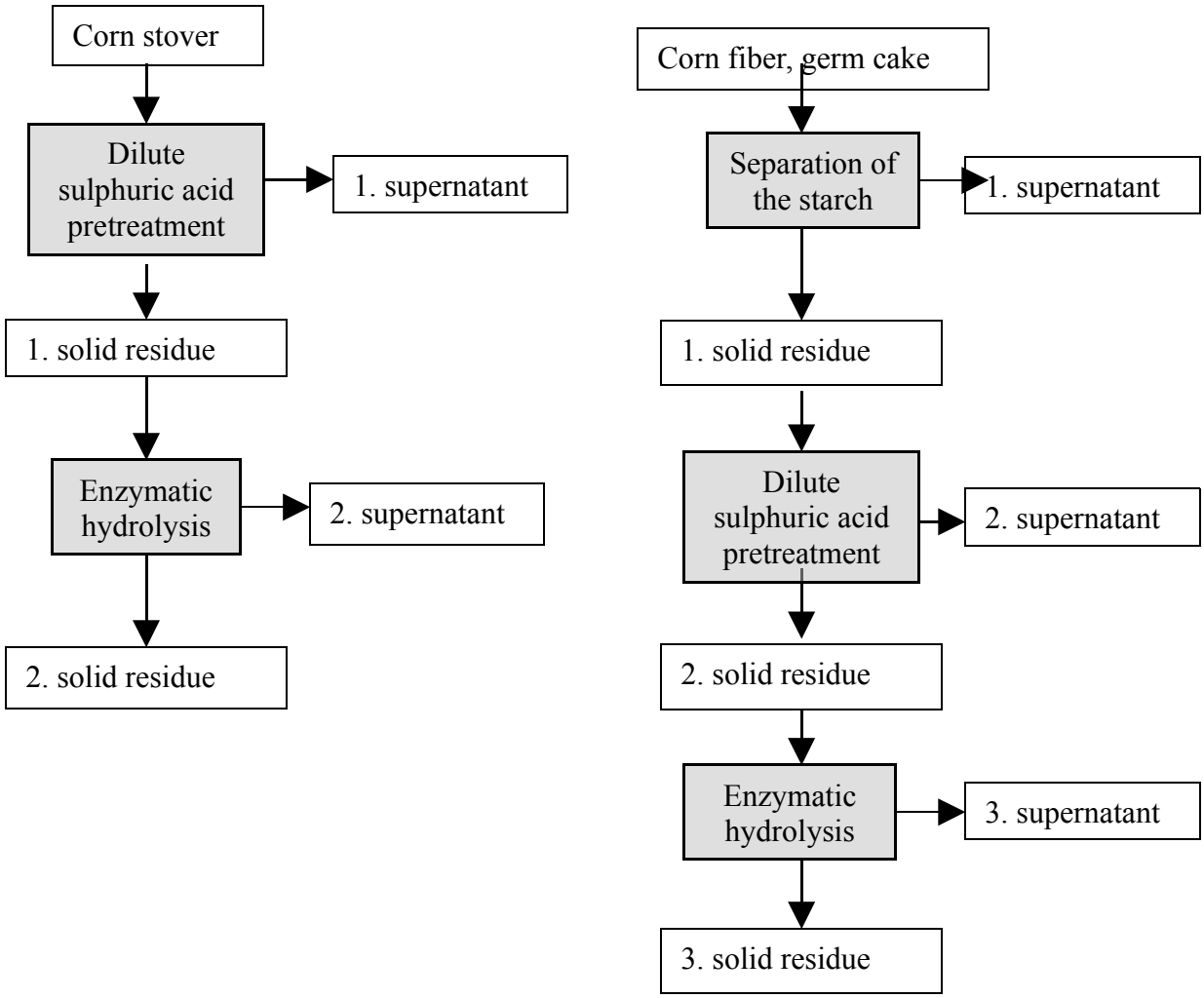
In the case of each raw material it was my goal to develop a fractionation process, which is able to sharply separate the fractions. The processes were aqueous treatments, followed by the separation and the analysis of the liquid and the solid phases. The treatments at the temperature of 120ºC were conducted in an autoclave, which was controlled by steam pressure. In the case of the corn stover the first process was a low temperature (T=120ºC) dilute (0.5-5% w/w) sulphuric acid pretreatment at long residence times (t=60-120 minutes). The severity of the pretreatments was featured by the combined severity factor (CSF).

$$\text{CSF} = \log \text{Ro-pH},$$

$$\log Ro = \log\left[t * \exp\left[\frac{(T_r - T_b)}{14.75} \right] \right],$$

Where:
log R_0: severity factor; t: time [minutes]; T: reaction temperature [ºC]; T_b: reference temperature (100ºC).

During pretreatments the dry matter content was 8-10%, no stirring was applied. In the case of corn fibre and germ cake prior to dilute acid pretreatment I extracted the starch. The starch was initially extracted by using enzymes (α-amylase, gluco-amilase), but my results proved that even clear water is capable of extracting the starch. The destarched, dilute acid pretreated corn fibre and germ cake, and the pretreated corn stover were submitted to enzymatic hydrolysis. I tested different enzyme dosages (5-30 FPU/g d.m.) and retention times (0-72 hours). The outline of the fractionations can be seen below.



4. *Results*

Corn stover was the substrate at numerous researches previously. The results of these researches prove that it is necessary to make value added products from the hemicellulose and the lignin as well, in order the lignocellulosic ethanol to be economical. There are many ways of utilizing the pentoses, so it is a priority to yield these components from the raw material just like to make its cellulose digestible. Considering the literature results I chose to survey the effect of low temperature (120ºC) dilute sulphuric acid pretreatment at long residence times (>60 minutes). Lowering the energy demand of the pretreatment can contribute to the amendment of the carbon balance of lignocellulosic ethanol. The acid concentrations I used were 0.5, 2 and 5% w/w, the residence times: 60, 90 and 120 minutes.

I ascertained that sulphuric acid at the concentration of 2% after 90 minutes detention time reached the sharpest separation. After this pretreatment 90% of the glucan remained in the solid fraction, while 81% of the pentosan fraction appeared in the supernatant. Dilute acid pretreatment at higher reaction temperatures does not result in such high pentose yields. The reaction parameters that I chose were sufficient to sharply separate the cellulose and the hemicellulose. I conducted 48h enzymatic hydrolyses to survey whether the extraction of the hemicellulose is enough to make the cellulose enzymatically digestible or not. The maximum glucose yield in the enzymatic hydrolyses was about 46% of the original glucan content of the raw material, which proves that the extraction of the hemicellulose is not enough to make the cellulose digestible. The decrease of cristallinity, the increase of surface area, and the change

of the lignin structure may be necessary for the further improvement of cellulose digestibility, which can be implemented in a second, different type of pretreatment before the enzymatic hydrolysis.

The corn fibre (the hull of the seed) arises during the wet mill process. This by-product – in spite of the corn stover – is available on the site of the corn processors, it does not need collection, so its utilization would be highly reasonable. The goal of my studies was to yield sugars, phytosterols, phytosterol esters out of the raw material. The gas chromatography measurements detected 7% sterol esters and 2% sterols in the corn fibre oil. These are extremely high values as compared to other edible oils.

The first step was to optimize the extraction of the starch. I proved that the starch can be extracted by using distilled water just like by using α-amilases. The aqueous extraction at elevated temperature (120ºC) presumably eliminated the endosperm particulates which were adsorbed to the aleuron layer. The starch content of the corn fibre can be suspended without the use of enzymes or chemicals, and it can further be processed, concentrated, hydrolysed. An aqueous extraction results in a colloidic suspension, as the starch is not soluble in water. Gluco-amylases can easily hydrolyze the suspended starch. The destarched solid residue contained the cellulose, the oil and the hemicellulose without any losses.

After the aqueous extraction I treated the solid residue with dilute sulphuric acid to extract the hemicellulose. The optimization of the dilute acid pretreatments proved that in the case of corn fibre sulphuric acid at the concentration of only 1% can sharply separate the hemicellulose and the cellulose. About 90% of the cellulose remains in the solid fraction, while 99% of the hemicellulose is extracted as monosaccharides. This means such a sharp separation between the two fractions, which was not published previously. Simultaneously, the corn fibre oil and the phytosterols remained in the solid residue without any loss.

I did enzymatic hydrolysis tests with the solid residue. I surveyed the effect of the elimination of the hemicellulose on the digestibility of the cellulose. I also tested the behaviour of the corn fibre oil and especially the phytosterols. I proved that an enzyme dosage of only 5 FPU/g d.m. plus 5 IU/g d.m. β-glucosidase activity is enough to fully convert the cellulose to glucose. These are especially low values, since other lignocelluloses need 15-30 FPU/g d.m. enzyme dosage for to be hydrolysed. I proved that the oil content of the fine fibre has not only remained in the solid residue, but the extraction of this residue yielded twice as much oil (4.8 g) than the direct extraction of the native corn fibre. This phenomenon, which was not reported previously, is presumably due to the release of bound lipids which are in the form of lipopolysaccharides and lipoproteins in the pericarp. The total sterol concentration (7%) was similar to those values detected in the native corn fibre oil. Thus the yield of these valuable compounds has also increase nearly two times, from 0.21 g total sterol to 0.38 g total sterol per 100 g corn fibre. Besides the release of bound lipids some more sterols and sterol esters became extractable, too. This phenomenon was not published previously. The oil extracted from the solid residue of the fractionation process was significantly different in terms of phytosterol composition than commercial corn oil (for example it contained significantly more Δ5-avenasterols.

For the further utilization of the sterols and sterols esters (for example to make functional foods) it is rational to separate them from the oil extracted at the end of the fractionation. The sterols and sterol esters can be yielded from the oil with a certain combination of saponification, extraction, distillation and crystallization. The residual oil can be utilized as

raw material for biodiesel or surfactant production or it can be mixed to feedstock or it can be burnt.

The analysis of each fractions lead to new establisments. The gravimetric determination of Klason lignin is biased if the sample contains significant amounts of proteins and/or lipids, as these components partially (or fully) remain in the precipitate. The patented method of starch measurement is biased if the sample contains hemicellulose, since the hemicellulose can contain some glucan as well. In this case the accurate determination of starch needs it's enzymatic hydrolysis.

The germ cake was tested when I already had the results of the corn fibre tests. Thus I used the optimal conditions of pretreatment, and tested, if the residual oil content (15%) of the germ cake can be concentrated in the solid residue, and whether this way the overall oil yield can be improved or not. The results proved that the concentration of the oil in the solid residue is possible, but the overall oil (and sterol) yield can not be increased. Simultaneously, the separation of the different polysaccharides was not as sharp as at the corn fibre. The method that I developed is not in every case suitable for the separation of starch, hemicellulose, cellulose and oil, and for the increase of oil yield. The optimal conditions of separating these fractions depend on the raw material.

5. Theses

1. Dilute sulphuric acid pretreatment of corn stover, corn fibre and germ cake (acid concentration: 1-2% w/w, reaction temperature: 120ºC, residence time: two hours) is suitable for the sharp separation of hemicellulose and cellulose. (*The extraction of the hemicellulose is not in every case enough to make the cellulose enzymatically accessible, see for example: corn stover. If the hemicellulose/cellulose ratio of the raw material is high, and the lignin content is low, then the extraction of the hemicellulose makes the cellulose enzymatically highly accessible, digestible, see for example: corn fibre.*) In the case of corn fibre 90% or higher conversion rates can be reached using only 5 FPU/g d.m. enzyme dosage. [*Kálmán, 2006a,b*]
2. Extracting the polysaccharide content of corn fibre and corn germ cake results in solid residues, the lipid content of which is higher than the lipid content of the raw material. (*In the case of corn fibre the increase can be twenty fold.*) Extracting the polysaccharide content of the corn fibre results in the significant increase of the overall oil and phytosterol yield vs. direct extraction of corn fibre oil from the raw material. (*Corn fibre oil yield was increased two times by using the fractionation process, while the phytosterol yield increased by about 80%.*) [*Kálmán, 2006a,b*; *Kálmán, 2008*]
3. The official method of measuring starch (based on a hydrochloric acidic hydrolysis at elevated temperature) is biased if the raw material contains hemicellulose. (*In this case for the accurate determination of starch enzymatic hydrolysis is necessary.*) [*Kálmán, 2006a*; *Kálmán, 2008*]
4. The determination of Klason-lignin is biased if the raw material contains significant amounts of lipids or proteins, as the case at corn fibre and corn germ cake. (*In this case it is necessary to separately determine the total (free and bound) lipid and protein contents.*) [*Kálmán, 2006a*]

6. Utilization

The low temperature (T=120ºC) dilute sulphuric acid pretreatment of the corn stover can gain significance if the value of the pentoses increase. This can be generated by the development of the effective fermentation of the pentoses, or the increase of the demand for other products (for instance bio-surfactants) which can be made from pentoses. The cellulose which is yielded through the low temperature dilute acid pretreatment is not accessible for enzymatic hydrolysis enough, so the production of cellulosic alcohol needs further pretreatment. However, this fibre which is not digestible may be a raw material of other applications (for example building industries, production of composites).

The corn fibre arises at one place at the corn processors, so it is rational to apply the fractionation method that is described in this paper in order to yield valuable products like sterols, sterol esters and monosaccharides. The solid residue of the fractionation, which contains high concentrations of corn fibre lipids, as a new product, is a patented new product.

The price of sterols and sterol esters – depending on the component – is between 10 and 50 euros per kg. The process described in this thesis yielded 0.38 g total sterols/100 g corn fibre, and it equals 38-190 euros per metric tons of corn fibre revenue. The fuel ethanol which can be fermented from the glucose has a wholesale price of about 0.8 euro per liter. Considering an average glucose yield of 31 g/100 g corn fibre it can be calculated that 158 kg (200 l) ethanol can be produced per metric tons of corn fibre, which means about 160 euros/t c.f. revenue. Altogether we can calculate 198-350 euros income from one ton corn fibre, and the possible value of the pentoses is still not considered. Corn fibre as a feedstock is marketed for about 72 euros per metric tons. This simplified calculation signs that the fractionation process described in this thesis may be economically feasible, since it demands mild reaction conditions and little amounts of reagents.

7. Publications

This thesis is based on the following publications:

1. **Kálmán, G.**, Gáspár, M., Recseg, K., Réczey, K., **2006**a: Novel approach of corn fiber utilization. Applied Biochemistry and Biotechnology, **129-132**, 738-750.
2. **Kálmán, G.**, Varga, E., Réczey, K., **2002**: Dilute Sulphuric Acid Pretreatment of Corn Stover at Long Residence Times. Chemical and Biochemical Engineering Quartely, **16**, 151-157.
3. **Kálmán, G.**, Réczey, K., **2008**a: Consecutive aqueous extractions of wet-milled corn germ cake. Chemical and Biochemical Engineering Quarterly.
4. **Kálmán, G**., Réczey, K., **2007**: Possible ways of bio-refining and utilizing the residual lignocelluloses of corn growing and processing. Periodica Politechnica, **51/2**, 29-36.

Other publications related to the issue:

1. **Kálmán, G.**, Réczey, K., Juhász, T., **2006**b: Lignint és rostolajat tartalmazó készítmény és eljárás annak előállítására. *Szabadalmi Közlöny és Védjegyértesítő*, P0400804. sz. szabadalmi bejelentés.
2. **Gáspár, M.**, Kálmán, G., Réczey, K., **2007**: Corn fiber as a raw material for hemicellulose and ethanol production. Process Biochemistry, **2007/42/7**, 1135-1139.
3. **Kálmán, G.,** Réczey, K., **2006**c: Gabonaipari melléktermékek alternatív hasznosítása az élelmiszer- és vegyiparban. Magyar Kémikusok Lapja
4. **Réczey, K.**, Gáspár, M., Kálmán, G., **2005**: Kukorica – másképpen. *Agrofórum* (extra – 9.) 72-74.
5. **Kálmán, G., 2006**d: A kukorica széleskörű ipari felhasználásának lehetőségei. *Agrofórum*, 17. 1/M. 17-20.

Presentations:

1. **Kálmán, G.,** Réczey, K., **2008**: Novel approach for the utilization of the by-products of bioethanol production from corn. ERA-Chemistry Workshop. Chemistry of raw material change, chemical transformation of biomass. 13-16'th April, 2008, Kraków, Poland. (Oral presentation)
2. **Kálmán, G.,** Réczey, K., **2002**: Kukoricaszár híg savas előkezelése hosszú tartózkodási idővel. KÉKI 307. tudományos kollokvium, Budapest. (Oral presentation)
3. **Gáspár, M.**, Sárdi, Á., Kálmán, G., Réczey, K., **2003**: Hemicellulóz B izolálása kukorica maghéjból. Műszaki Kémiai Napok, Veszprém.
4. **Gáspár, M.**, Kálmán, G., Réczey, K., **2005**: Két út a kukoricarost hasznosítására. Műszaki Kémiai Napok, Veszprém.
5. **Kálmán, G.**, Réczey, K., **2006**: Új lehetőség a kukoricarost hasznosítására. Műszaki Kémiai Napok, Veszprém. (Oral presentation)