Verbesserung der Energie-, Stoff- und Emissionsbilanzen bei der Bioethanolproduktion aus nachwachsenden Rohstoffen

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Twenty years of trials, tribulations and research progress in bio-ethanol technology

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List of abbreviations

% (w/w)	-	weight percent (weight/weight)
°C	-	specific temperature on the Celsius scale
316SS	-	special stainless steel
a	-	abbreviation for year
Å	-	Ångström (unit of lenth)
bar	-	unit of pressure
C_5	-	furanose sugar
C_6	-	pyranose sugar
CBHI	-	cellobiohydrolase (working from the reducing end of cellulose)
CBHII	-	cellobiohydrolase (working from the non-reducing end of cellulose)
CH_4	-	methane
CO_2	-	carbon dioxide
DP	-	number of sugar molecules
DS	-	dry substance
E 85	-	mixture of 85% dehydrated ethanol and normal fuel
e.V.	-	registered association
E10	-	mixture of 10% dehydrated ethanol and normal fuel
EC	-	enzyme commission number (numerical classification scheme for enzymes)
ETBE	-	ethyl tertiary butyl ether
FNR	-	Fachagentur Nachwachsende Rohstoffe
FPU		Filter paper units (equals the amount of enzyme that is necessary to hydrolyse 1µm/min of glycosidic bonds on Whatman#1 filter paper under special conditions)
FS		Fermentable substance is the amount of carbohydrates that can be yielded after an enzymatic hydrolysis over night, using an overdosed enzyme dosage (in relation to the weight in) [%]
GABI	-	Software for integrated balancing
Gammaclast 2OP	-	Alpha-amylase from Aspergillus *spec.* (Gamma Chemie, Darmstadt, Deutschland)

GC 106	-	Low pH protease for protein hydrolysis (Genencor, Leiden, The Netherlands)
GC 220	-	cellulase enzyme complex (Genencor, Leiden, The Netherlands)
GC 626	-	acid alpha amylase (Genencor, Leiden, The Netherlands)
GC 880	-	cellulase enzyme complex (Genencor, Leiden, The Netherlands)
h	-	hours
H_2SO_4	-	sulphuric acid
H_3PO_4	-	phosphoric acid
HCl	-	hydrochloric acid
HMF	-	hydroxymethyl fufural
l	-	litres
LCA	-	Life Cycle Assessment
LCI	-	Life Cycle Inventory
Liquozyme 280L	-	Thermostable bacteria amylase from Bacillus licheniformis (Novozymes, Bagsvaerd, Denmark)
MJ	-	energy unit
NaOH	-	sodium hydroxide
NREL	-	National Renewable Energy Laborator
Optimash BG	-	hemicellulase enzyme complex composed of a ß-glucanase/ xylanase-mixture (Genencor, Leiden, The Netherlands)
pH	-	a measure of the acidity or alkalinity of a solution
ROZ	-	octane number
rpm	-	revolutions per minute
SAN Super	-	Alpha-amylase/amyloglucosidase/protease enzyme complex (Novozymes, Bagsvaerd, Denmark)
SBD	-	starch binding domain
SHF	-	separate hydrolysis and fermentation process
SO_2	-	sulphur dioxide
SSF	-	simultaneous saccharification and fermentation process
STARGEN 001	-	enzyme mixture of an Aspergillus kawachi alpha amylase expressed in Trichoderma reesei and a glucoamylase from Aspergillus niger to hydrolyse granular starch (Genencor,

Leiden, The Netherlands)

Introduction

The global climate is affected by the use of fossil fuels which cause the carbon dioxide that has been stored for millions of years to be released into the atmosphere (ÖHGREN et al, 2006). Additionally, fossil fuels are a finite energy source. Thus, there is an escalating pressure to develop alternative nonoil-based sources of energy (CHEN et al., 2006). One promising approach is to produce bio-ethanol, at first as an additive to normal fuels (up to 85%) and maybe later on as a substitute for fossil fuels. The production of ethanol is vindicated by its potential to reduce greenhouse gases (SENN und LUCÁ, 2002) in particular CO_2 in traffic and transportation respectively. Theoretically bio-ethanol can be produced from any biomass, thus the access to raw material is only limited by the area of farmland that can be used for biomass production, and provides nonoil-producing countries to be more independent in fuel. Additionally, biomass is the only foreseeable sustainable source of materials for biofuels production available to mankind (LYND et al., 1999).

Today ethanol is produced from sugar beet, sugar cane or starchy materials. The by-products of these agricultural materials mainly remain unused for energy production. If, for example, the straw resulting from the cultivation of cereals could be recovered for ethanol production, the potential of dry biomass used for ethanol production could be doubled. During the world wars, when crude oil was in short supply, scientists focussed on the conversion of cellulosic biomass to ethanol. In those days acid hydrolysis processes, which caused corrosion problems to the production plants, were used. The beginning of such working methods has been known from the wood saccharification since the early 19th century (FEITLER, 1903; ROEMPP, 1995). For the past decade biomass originated fuels such as ethanol have gradually gained increasing attention and efforts have been made to produce them from lignocellulosic biomass like wood, straw or corn stover in an environment friendly manner.

Objective

Until now, no really large distillery for processing lignocellulosic material has been built worldwide. Some companies have merely built bigger pilot plants. Only starchy materials such as maize and other cereals (wheat and triticale) or sugar cane are used to produce bio-ethanol in large industrial distilleries. Different processes could be used for producing bio-ethanol from starch. For example the high-pressure steam process which uses a so-called “Henze-Dämpfer”. A “Henze-Dämpfer” is a cylindric-conical steam cooker that works at temperatures over 100°C at a pressure of 4-6bar. Alternative processes work without overpressure at temperatures under 100°C. Such processes are, for example, the mill-and-mash process and the dispersing-mash process. Here the starchy raw material is milled using a roller mill and a hammer mill respectively or the raw material is dispersed using a rotor-stator system before the starch is digested with enzymes to fermentable sugars (KALTSCHMITT and HARTMANN, 2001). One such process, the Hohenheimer dispersing-mash-process (SENN, 1988) with stillage recycling (PIEPER and JUNG, 1982), is a very simple and profitable process for the conversion of starch into ethanol. This well-investigated and well-balanced process is the basis for an energy and cost balance study appointed by the “Bundesverband landwirtschaftliche Rohstoffe verarbeitende Brennereien e.V. unter Beteiligung des Bundesverbandes Deutscher Kartoffelbrenner e.V.“ led by SENN in 2002. This study showed that it is possible to produce ethanol in smaller regional distilleries for nearly the same price as in large industrial distilleries. Additionally, this study took a look at the possibility of using the cellulosic material from the agricultural crop rotation and the stillage from the distillery process to produce biogas with the aim of improving the energy yield of the whole process and designing a sustainable ethanol process. As mentioned above, the additional use of the resulting cellulosic material doubles the dry substance content that can be harvested and, therefore, increases the potential to produce sustainable energy. The aim of this work is to develop a process using starchy and cellulosic materials in one distillery to increase the ethanol yield of the process, improve the energy output of the process and to create an ecobalance for this process.

1 Preface

1.1 Ethanol as renewable fuel

1.1.1 Why ethanol?

First of all there is the question: “Why is it useful to produce ethanol as an alternative fuel from renewable biomass?” The answer is that ethanol is very attractive as an alternative fuel, because it produces a much lower greenhouse gas output than fossil fuels. Additionally, ethanol makes it possible to fulfil the EU-objective of substituting 20% of the EU fossil fuel consumption with CO_2-neutral fuels by 2020. Adding ethanol to normal fuel produces some advantageous physical properties. It is easy to blend ethanol (99.85%mas) into normal fuel in a low level blend with 10% dehydrated ethanol and 90% normal fuel, the so-called E10. This mixture can be used in most unmodified vehicles as they are built today (VDA, 2008). Moreover ethanol also can be blended with fossil fuel to give high-level gasoline blends of up to 85% dehydrated ethanol and 15% normal fuel, the so-called E85. In order to use such a mixture as a fuel, the vehicles have to be modified to so-called flexible fuel cars that can be driven with different mixtures of dehydrated ethanol and normal fossil fuel up to 85% of ethanol in the mixture. Thus it is possible to drive such cars with normal fuel or to use a mixture of ethanol and normal fuel up to 85% dehydrated ethanol. Today a lot of such cars have already been produced and registered in Brazil (ANFAVEA, 2008; MORRIS, 2006). One big advantage of blending nearly anhydrous or dehydrated ethanol to normal fossil fuel is the high octane number of ethanol (114 ROZ). Normal fuel only has an octane number of 92 ROZ. After ethanol has been added to the fossil fuel, no further additives, so-called octane boosters, which usually are very toxic, are needed. For example, E85 has an octane number of 109 ROZ and, therefore, a much higher octane number than normal fossil fuel (LEE et al, 2007). Adding ethanol to the fuel improves the combustion of the fuel in the motor, because adding ethanol to the fuel also adds a lot of oxygen. Consequently, the tail pipe CO-emissions can be reduced (POULOPOULOS et al., 2001; HSIEH et al, 2002; YÜCESCU, 2006). However, adding ethanol to normal fuel reduces the energy

content per litre, because ethanol only contains 21.2MJ/l instead of 31.0MJ per litre of fossil fuel. Thus the mixture of ethanol and fossil fuel (E85) contains ca. 22.7MJ per litre. Another way of adding ethanol to gasoline is the production of ethyl tertiary butyl ether (ETBE), which provides the same benefits as blending fossil fuel with dehydrated ethanol.

1.1.2 Today's world ethanol production

The growing dependence of the transport sector on oil and the rising world oil price and energy consumption as well as the commitments of the Kyoto Protocol to reduce emissions have lead to a worldwide boom for bio-fuels. Brazil, which was the world biggest ethanol producer for years (fig. 1.1.1), already started a state-run programme for ethanol production in the 1970's. The aim of this programme was to increase the amount of biogenous fuels in traffic. Similarly the USA as today's world biggest ethanol producer (fig. 1.1.1; RFA, 2008) also had some state-supported subsidy programmes for bio-ethanol in the last decades. So similar to Brazil, the production of bio-ethanol in the USA is supported by the national government, enabling tax reliefs. Today the worldwide production of ethanol could reach the mark of 50 billion litres of ethanol (GATTERMAYER, 2006). Compared to the 44.9 billion litres of ethanol produced in 2005 (fig. 1.1.2), this is an increase of ca. 10%.

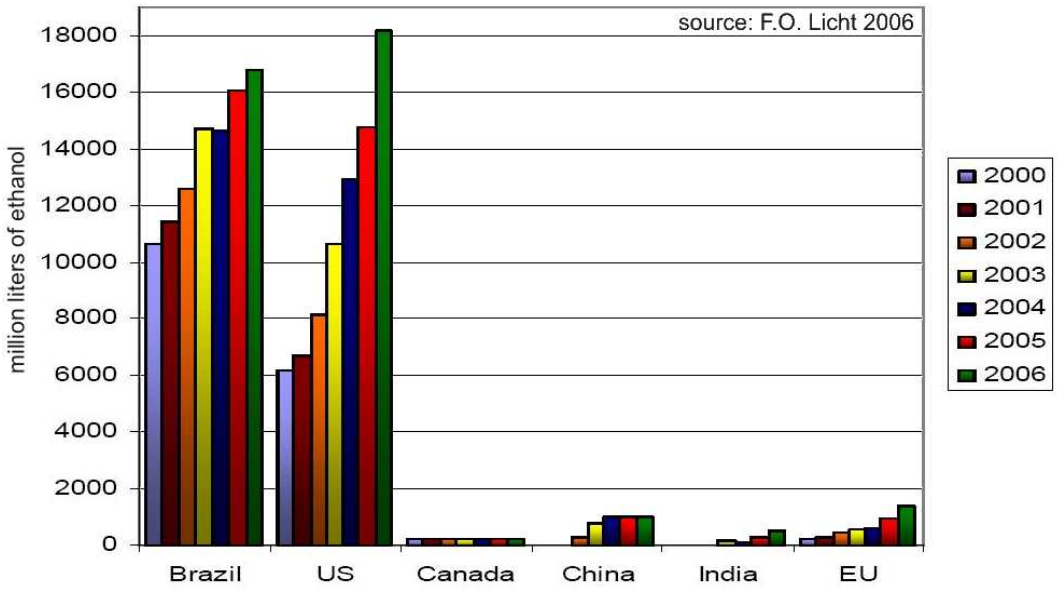


Fig. 1.1.1 The world ethanol production by country (F.O. Licht, 2006)

Because of the aims of the EU to reduce the greenhouse gas emissions by using biofuels, a lot of different companies, for example Südzucker and Sauter in Germany, started to erect large ethanol plants. These plants may contribute to the big boom of ethanol in Europe. In addition to those already operating ethanol plants further projects are planned. Fig. 1.1.3 shows where new projects in Germany are to be realized and their capacity.

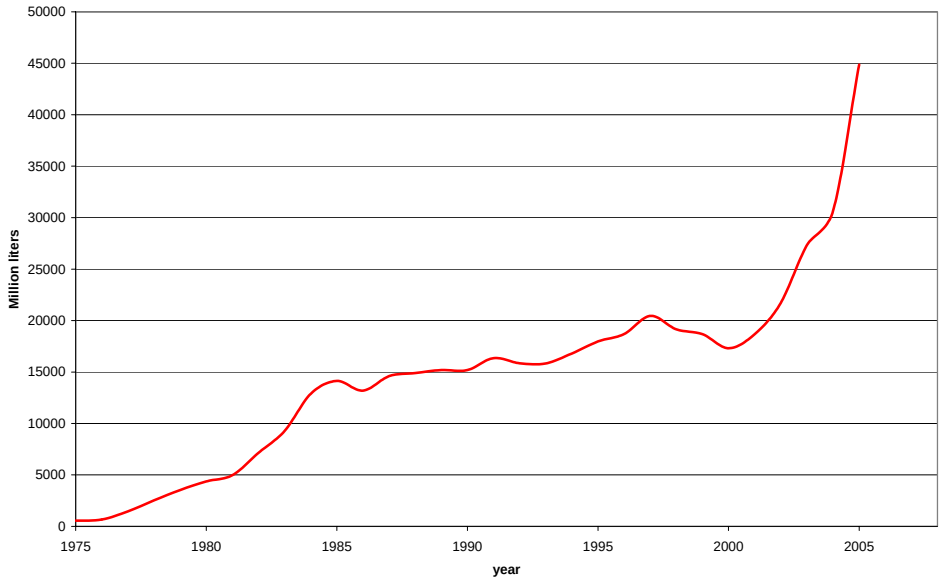
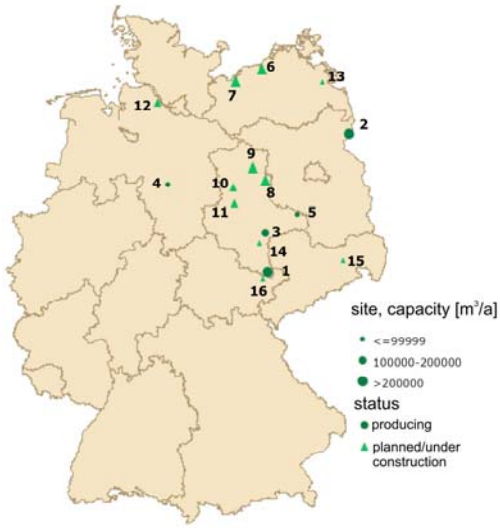


Fig. 1.1.2 The world ethanol production from 1975 to 2005 (Source: Hunt, S. and Stair, P., Worldwatch Institute, 2006)

Another country that produces a lot of ethanol in Europe is France. But France lags behind other European countries adding ethanol to other fossil fuels.



c	company	site	capacity[m³/Jahr]
producing plant			
1	Südzucker Bioethanol GmbH	06712 Zeitz	260000
2	NBE - Nordbrandenburger BioEnergie GmbH & Co. KG	16303 Schwedt/Oder	225000
3	MBE - Mitteldeutsche BioEnergie GmbH & Co. KG	06780 Zörbig (Bitterfeld)	100000
4	KWST Kraul & Wilkening und Stelling KG - GmbH & Co.	30559 Hannover	20000
5	Bernhard Icking KG	06918 Seyda (Wittenberg)	7500
plant under construction			
6	Abengoa Bioethanol	18147 Rostock	370000
7	VERBIO Vereinigte BioEnergie AG	23966 Wismar	253165
8	ECOPOWER BIOFUELS AG	39307 Genthin	238000
9	BioEthanol Arneburg Gmbh & Co. KG	39576 Stendal	202530
10	Anlage Bülstringen	39345 Bülstringen	200000
11	Nordzucker AG	39164 Klein Wanzleben	130000
12	PROKON Nord Energiesysteme GmbH	21682 Stade	100000
1	Südzucker Bioethanol GmbH (Ausbau)	06712 Zeitz	100000
13	Danisco Sugar GmbH	17389 Anklam	60000
14	Heuß GmbH	06118 Halle (Saale)	40000
15	Unternehmensgruppe Müllermilch	01454 Leppersdorf	10000
16	WABIO Bioenergie	07586 Bad Köstritz	8400

Fig. 1.1.3 Germany's ethanol plants (FNR, 2007)

2. Theoretical basis

2.1. Raw materials for the production of bio-ethanol

Various raw materials could be used for the production of ethanol. Theoretically any biomass that contains fermentable sugars, such as glucose, galactose and mannose (BOLES, 2006), can be used to produce ethanol. However, before the sugars can be fermented to ethanol by normal yeast cells, the structure of the biomass has to be disrupted and hydrolysed to liberate fermentable sugars, because usually the sugars are stored as storage polysaccharides (DA ROSA, 2009).

2.2. Starch containing raw materials

2.2.1. Composition of starchy materials

Today in Europe and North America mostly starchy materials are used to produce ethanol. These include potatoes, wheat, maize and rye respectively, which are used to produce technical or drink alcohol. To process those materials the starch, a water insoluble and non-fermentable storage polysaccharide, has to be hydrolysed to glucose. Starch is a glucose-based polymer that consists of amylose (fig. 2.2.1, a linear α-1,4-bounded glucose chain) and amylopectin (fig. 2.2.1, a linear α-1,4-bounded glucose chain with α-1,6-branches of glucose) (BEYER, 1998). The ratio between amylose and amylopectin varies depending on the starch source (ZOEBERLEIN, 2001). In plants starch occurs, in granules which are visible microscopically. The size of the granules and how they look like is different and depends on the starch source (CZAJA, 1969; GALLANT et al, 1972; FANNON et al, 1992, CHITONELLI et al, 2002). To make the starch chains accessible for enzymes, normally gelatinization of the starch must occur to weaken intra- and inter-molecular hydrogen bonds between the polysaccharide chains. After the starch has gelatinized and becomes accessible for an enzymatic digestion, technical enzymes, produced by bacteria or moulds, are added to hydrolyse the structure of the starch. In distillery processes using technical

enzymes, first of all an α-amylase is used. This enzyme cuts the α-1,4-bonds of the starch inside of the linear chains (SENN, 2001). The more scientifically correct or systematic name for this type of enzyme is 1,4-α-D-glucan glucanohydrolase and its EC number is 3.2.1.1 (RICHARDSON et al, 2002). The products of the α-amylase are so called dextrins and the action of the enzyme is random (KNIEL, 2000). The resulting dextrins are shorter sugar chains which in difference to the original starch are water-soluble oligosaccharides. α-amylase, as a starch hydrolysing enzyme, can only cut the α-1,4 bonds but not the α-1,6 bonds of the branches of the amylopectin (SENN, 2001). The next stage of starch hydrolysis to be performed is the saccharification phase. This phase is effected by another amylolytic enzyme called glucoamylase (SCHMITZ, 2003; SENN, 2001).

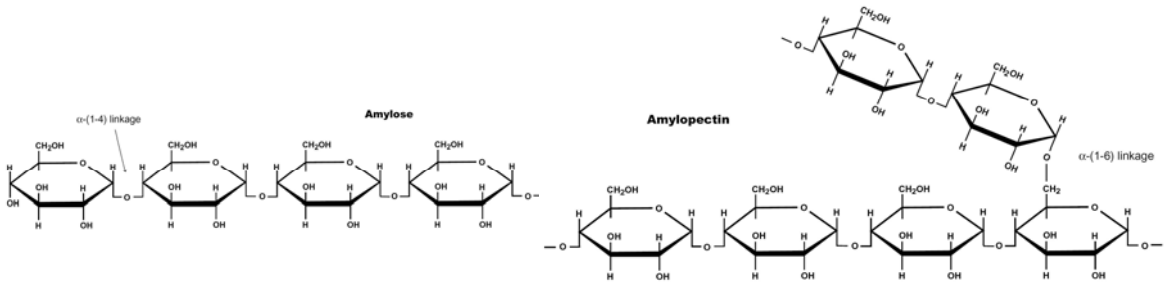


Fig. 2.2.1 Chemical structure of amylose and amylopectin

Enzyme	EC number	Source	Action
α-amylase	3.2.1.1	*Bacillus species*	A-1,4-linkages are hydrolysed to produce α-dextrins, maltose and oligosaccharides (DP3) or higher
		Aspergillus oryzae, Aspergillus niger	A-1,4-linkages are hydrolysed producing α-dextrins, maltose and oligosaccharides
Glucoamylase	3.2.1.3	*Aspergillus species, Rhizopus species*	A-1,4 and α-1,6-linkages are hydrolysed from the non-reducing ends, to yield glucose

Table 2.2.1 Technical enzymes used in the starch hydrolysis process

The main purpose of glucoamylase, which is also known as amyloglucosidase (glucan 1,4 –α-glucosidase; EC Number 3.2.1.3) is to hydrolyse terminal 1,4-linked α-D-glucose residues successively from the non-reducing ends of the dextrin chains. This causes the release of the fermentable sugar glucose. In addition to the α-1,4-bonds, some glucoamylases can also hydrolyze α-1,6-bonds. But these bonds are hydrolysed at a rate that is approximately 20 to 30 times slower than the

hydrolysis of the α-1,4-bonds. Technical glucoamylases are isolated from fungal sources such as *Aspergillus niger* and *Rhizopus species.*

2.2.2. The Stargen™ process

In contrast to the normal enzymatic starch hydrolysis processes, the stargen™ process does not use a gelatinization of the starch. For the stargen™ process temperatures below the point of gelatinization are applied. Table 2.2.2 shows the gelatinization ranges of some cereals. Hydrolysing the starch without gelatinization means that granular starch has to be hydrolysed. Common technical amylolytic enzymes of the *Aspergillus niger* species, that are used in distillery processes, are unable to hydrolyse granular starch. Similar to processes using the gelatinization of starch, the stargen™ process also uses α-amylases and glucoamylases. For the stargen™ process, however, a blend of starch hydrolyzing α-amylase from *Aspergillus kawachi* and glucoamylase from *Aspergillus niger* is used. These enzymes, other than common amylolytic enzymes, have a so-called starch binding domain (SBD) (fig. 2.2.2), which makes it possible for the enzymes to bind directly to the starch during its hydrolysis. This SBD is connected via a linker to the catalytic domain. This breaks down the starch to smaller starch oligomers which then can be hydrolysed by the glucoamylase.

Cereal	Gelatinization range [°C]
Maize	62-72
Barley	52-59
Rye	57-70
Rice (polished)	68-77
Sorghum (milo)	68-77
Wheat	58-64

Table 2.2.2 Gelatinization temperature ranges of various cereals. (Kelsall, D.R. and Lyons, T.P., 2003)

Using a combination of an α-amylase and a glucoamylase the starch can be hydrolysed directly to fermentable sugars. The two hydrolysis steps that are needed for this direct hydrolysis to fermentable glucose are, on one hand the exo-activity of the glucoamylase, which enables the drilling of sharp and deep pinholes

into the granular starch, and, on the other hand, the endo-activity of the alpha amylase, which leads to the widening of these pinholes. These combined activities continuously release fermentable glucose from the granular starch.

Fig. 2.2.2 Alpha-amylases with starch binding domain (SBD) (Konieczny-Janda, G and Shetty, J., 2005)

Applying the stargen™ process and, thereby, processing without gelatinization makes it necessary to ferment at a pH of approx. 3.2-3.5 so that the growth of infecting bacteria is inhibited. At these conditions, however, the cereals proteases are no longer active. Therefore an acid tolerant protease that produces enough free amino nitrogen (FAN) for the yeasts growth and metabolism has to be used. During the stargen™ process the digested starch is fermented in a so-called simultaneous sacharification and fermentation (SSF) process. Here the level of fermentable sugars is very low throughout the process because, as soon as sugar is liberated, the yeast transforms it directly to ethanol.

2.2.3. Processes for the production of ethanol from starchy materials

For the production of ethanol from starchy materials different processes can be used. The cereals containing the starch can be milled using a dry milling process or a wet milling process. To visualize the differences of these two process strategies the separate process flows have to be described.

2.2.4. The dry milling process

First of all, when using the dry milling process the grains must be milled to open the cereals structure. Most commonly a hammer mill is used for this process. When all grain is milled, the dry grists are mixed together with water and enzymes to the so called mash. This process is so called slurrification. Here also the pH is adapted to the optimum of the enzymes. As already described above, in the next steps called the liquefaction and the saccharification the starch is digested to

fermentable sugars. Then yeast and nutrients are added before the fermentation starts. At the end of the fermentation, during which the tank is cooled using a plate heat exchanger in a by-pass, the ethanol is liberated from the so called beer by distillation. The stillage that remains at the bottom of the distillation column at the end of the distillation process usually is divided into thin stillage that can be used as backset for the process and the thick stillage that is dried to produce DDGS (distillers dried grains with solubles). A part of the backset can be used as process liquid for the next mash and is mixed with new dry grits and water. This procedure is also used in other processes where it is called stillage recycling. The rest of the thin stillage is evaporated and mixed together with the dry solids before the drying process.

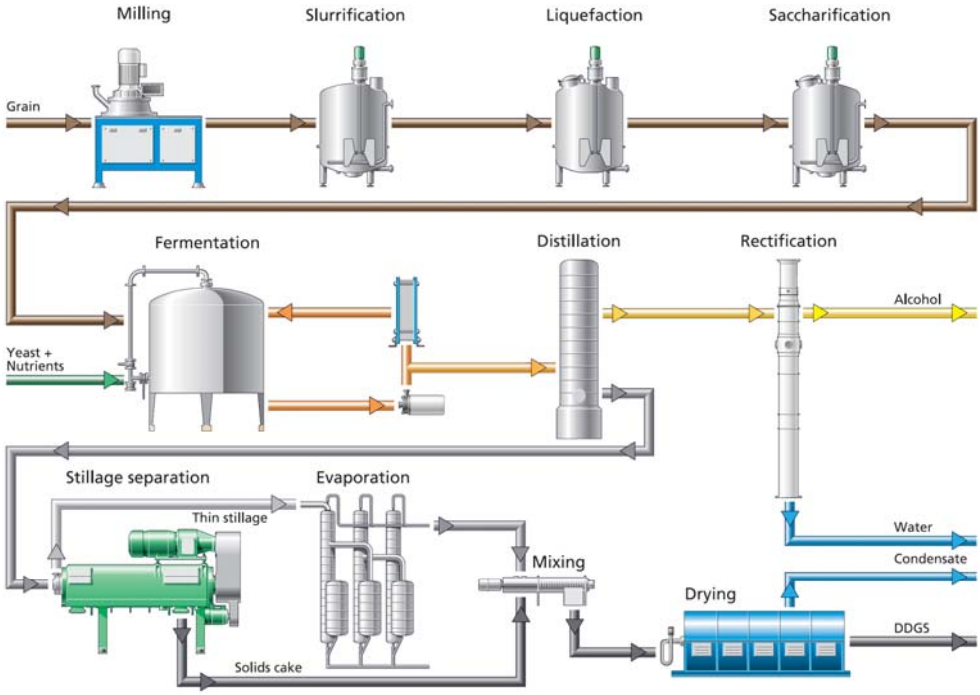


Fig. 2.2.3 Dry milling process for the production of ethanol from grain Source: Westfalia-Separators-Online (2009)

2.2.5. Wet milling processes

The dispersing mash process

In difference to the dry milling process, for the dispersing mash process no hammer mill but a so called rotor-stator-system, comparable to the system of the

Becomix DH 1200 that will be described in chapter 2.7, is used to crush the grain. Here the grain is mixed with water or stillage as a backset and is reduced in size. When using a rotor-stator-system the mash directly leaves this system.
After the size reduction of the raw material, similar to the dry milling system, the mash is digested in a mashing vessel using different enzymes (α-amylase and glucoamylase) at different temperatures at their optimal pH during the liquefaction and saccharification, respectively. Like for all ethanol processes, the sugar slurry is mixed together with yeast and nutrients to transform the sugars to ethanol. After complete fermentation the slurry is distilled in a mash distillation column to liberate the ethanol from the mash. If needed, the concentration of the ethanol water mixture can be increased in a rectification column. In industrial distilleries the stillage that leaves the mash column at the bottom is separated similar to the dry milling process to get thin stillage as backset and, after an additional evaporation and drying to produce the DDGS. In small local distilleries the separation of thin and thick stillage is performed using a settling vessel.

The wet milling process with gluten separation

In difference to the dry milling process and the dispersing mash process described above, not only ethanol and DDGS as by-products can be produced in modern dry milling processes. Beside these products in a modern ethanol plant vital gluten can also be produced to increase the potential of products and to help to make an ethanol process from grains more profitable. In such a modern wet milling process first e.g. wheat as a possible raw material is mixed with water and matured in a maturing vessel for 10-20h (not shown in fig. 2.2.4) to swell the inner structure of the wheat. After that the wheat is milled in an air-turbulence mill (e.g. “Ultra Rotor” - Jäckering, Hamm or “Turbofiner” - Pallmann, Zweibrücken) or in a roller mill to a particle size of < 240µm. After the milling step the bran of the raw material is separated in an air classifier (sifter) to bran and flour. To increase the starch yield in this process the bran is cleaned off from the sticking starch in a bran finisher. The liberated starch is transferred to the flour that was separated before in the finisher. The bran directly can be sold as a separate product. The alternative way to sell the bran is to pelletise them before selling.

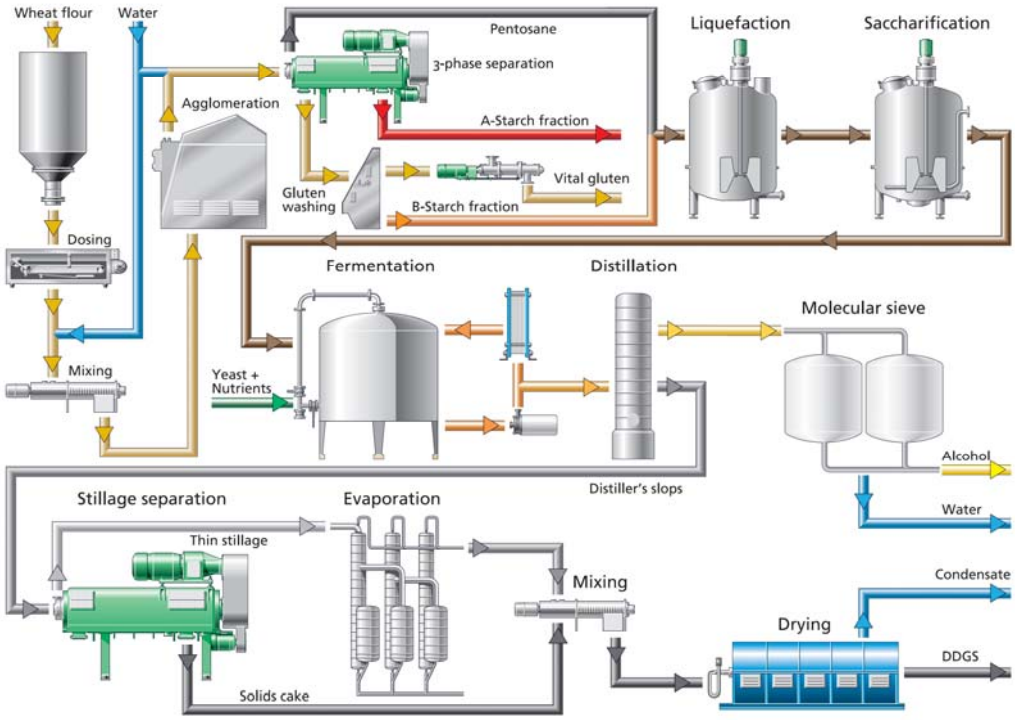


Fig. 2.2.4 Modern dry milling process for the production of ethanol from grain Source: Westfalia-Separators-Online (2009)

The flour is further processed in the production process. In a next step the protein of the raw material is to be separated from the starch and the other sugars (e.g. pentosans, cellulose, hemicellulose). To achieve this, first of all water is added to the flour to get a low viscosity dough. This dough is separated to a pentosan fraction, a starch fraction and the gluten fraction in a three phase decanter. To get high quality vital gluten the gluten is washed on a sieve and dried in a ring dryer to get a stable product with a long shelf life for the world gluten market. The other fractions (pentose fraction and starch fraction) can be combined and transferred to the starch liquefaction and saccharification process. After the digestion of the starch to fermentable sugars, similar to other ethanol production processes, yeast and nutrients are added for fermentation. After the end of the fermentation the alcoholic beer is transferred to the distillation where the ethanol is separated from the solids. If dehydrated ethanol is needed as end product the ethanol water mixture can be dried in a molecular sieve. The stillage that remains in the distillation column, similar to the dry milling process, is processed to get DDGS.

2.3. Lignocellulosic biomass

All lignocellulosic materials refer to plant biomass and are the most abundantly available carbohydrate polymer in nature (FENNEMA, 1996; BEYER, 1998). Their main component is cellulose (SAHA, 2005). Thus cellulose is an attractive raw material for the production of alternative energy. Additionally, lignocellulosic biomass can easily be replenished continuously by photosynthesis.

Lignocellulosic biomass comes in many different types, which may be grouped into four main categories:

- wood and wood residues (including sawmill and paper mill discards)
- municipal paper waste
- agricultural residues (including corn stover, sugarcane bagasse and straw)
- energy crops (e.g. energy corn).

In nature the degradation of lignocellulosic biomass is catalyzed by enzymes of fungi and bacteria and is a very slow process (FAN et al, 1987). This is due to the structural composition of the biomass and the low activity and degradation velocity of natural cellulase enzyme complexes. For an economic and effective biomass hydrolysis process a faster hydrolysis of the biomass and mainly a faster hydrolysis of cellulose is necessary. In the following section the natural structure of lignocellulosic biomass shall be described. Through this it is possible to explain the resistance of the lignocellulosic biomass and to understand the occurrence of problems for the digestion of the lignocellulosic biomass.

2.3.1. The composition of cellulosic biomass

Cellulosic materials are composed of three major components, extraneous substances (like terpenes, resins or phenols), polysaccharides and lignin. The polysaccharide components comprise high molecular weight carbohydrates, namely, cellulose and hemicellulose, which e.g. amount 60 to 80% for wood. In biomass cellulose is the major component of the cell walls. Cellulose (fig. 2.3.1) is

a linear β-1,4-bonded chain (BEYER 1998) of glucose and builds the smallest structural unit of the cellulosic biomass, the elementary fibrils. Cellulose is a non-water-soluble polysaccharide constructed of cellobiose which is built of two β-1,4-bonded molecules of glucose. Usually 500 to 5000 glucose units are connected together linearly (RICHMOND, 1990). The free rotation of the glycosidic bonds is prevented through intermolecular hydrogen bonds.

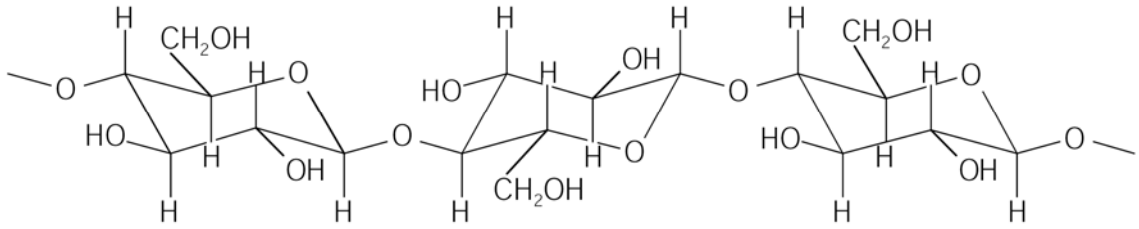


Fig. 2.3.1 The cellulose chain

These bonds between the 3-hydroxy-groups and the oxygen-atoms in the rings of neighbouring glucose-rests result in an immobilization of the macro-molecule and a nearly similar orientation of all glucose molecules. These regions in which the linear molecules of cellulose are bonded laterally are called crystalline regions. But not all cellulose is oriented in crystalline regions. Also paracrystalline and amorphous regions respectively can be found. Due to intermolecular hydrogen bonds between parallel oriented chains, a number of 60-70 cellulose chains are aggregated together, into a long slender bundle called microfibril (FAN et al., 1987) (fig. 2.3.2). These microfibrils are surrounded by polyoses such as hemicellulose and are cemented together to so-called fibrils. They are surrounded by a lignin and polyose layer which protects the fibrils from hydrolytic degradation (KUGA and BROWN, 1990). To enable a fast and almost total enzymatic hydrolysis of the material, pre-treatment is essential to degrade the protective layer of lignin and polyoses from the material, thus making the material more accessible for the enzymatic hydrolysis.

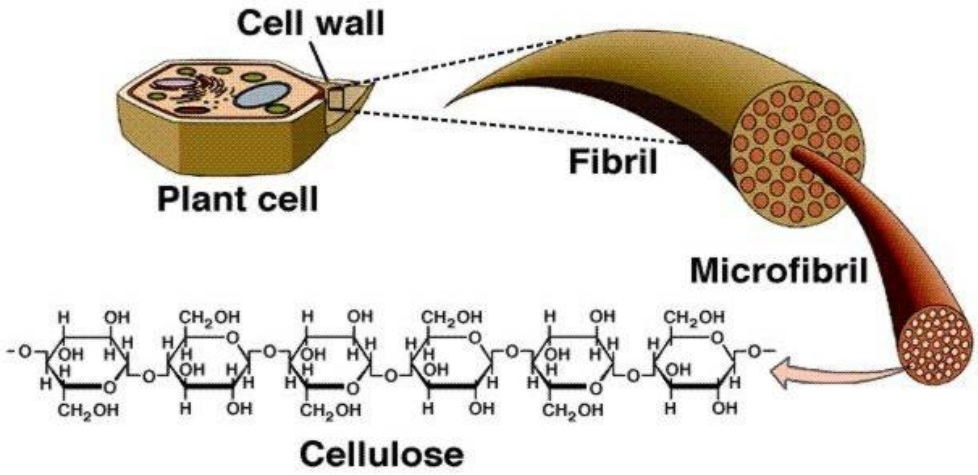


Fig. 2.3.2 Arrangement of fibrils, microfibrils and cellulose in cellwalls (source: Randy Moore, Dennis Clark and Darrell Vodopich, Botany Visual Resource Library © 1998 The McGraw-Hill Companies, Inc.)

For a better understanding of the properties and the behaviour of the non-cellulose components they are described in the following.

Hemicellulose

Hemicellulose is a group of polysaccharides with different compositions, which appears in plant fibres and cell walls of gramineous and grain. Often hemicellulose is associated with lignin and cellulose as mentioned above. Hemicellulose consists of different types of monosaccharides which are linked together glycosidically (CHO et al, 1999). These glycosidic chains are cross-linked and the degree of polymerization varies between 50 and 250. In hemicellulose different monosaccharides such as galactose, glucose and mannose can be found as hexoses. Additionally, xylose and arabinose appear as pentoses. Hemicellulose is amorphous because of its wide branching and can be solubilised in alkaline, diluted acids and partly in water.

Lignin

Lignin is a chemical compound that is most commonly derived from wood (BEYER, 1998) and is an integral part of the cell walls of plants. It is one of most common organic compounds on earth like cellulose (FUCHS, 2007) and chitin. As mentioned above, lignin fills the spaces in the cell wall between the cellulose microfibrils and hemicellulose. Lignin forms covalent bonds to polysaccharides and

thereby cross-links the biomass polysaccharides (WYMAN, 1996-A). Lignin is indigestible by mammalian and other animal enzymes. Only certain fungi are able to biodegrade the polymer. But to date the details of the reaction scheme of the biodegradation of lignin are not fully understood. Because of its cross-linking with the other cell wall components, it minimizes the accessibility of cellulose and hemicellulose to microbial enzymes. Lignin is a large, cross-linked macromolecule with molecular mass in excess of 10,000 atomic mass units. It is relatively hydrophobic and aromatic in nature. Fig. 2.3.3 shows the structure of a small piece of the lignin polymer. Lignin is a three dimensional phenyl propane polymer (FAN, 1987; BURGERT et al, 2006; DA ROSA, 2009) with phenylpropane units held together by ether and carbon bounds (FORSS and FREMER, 1975). The most abundant phenylpropane units in lignin are the 4-hydroxyphenylpropane, guajacylpropane and the syringylpropan. Because of its connection to the carbohydrates lignin gives structural rigidity. Because of its chemical composition, lignin acts like a “waterproof” coating around the cellulose fibrils. This is a useful property for the transport of water in the plant cells but, with regard to the digestion of biomass, the lignin is an obstacle for easy biomass degradation.

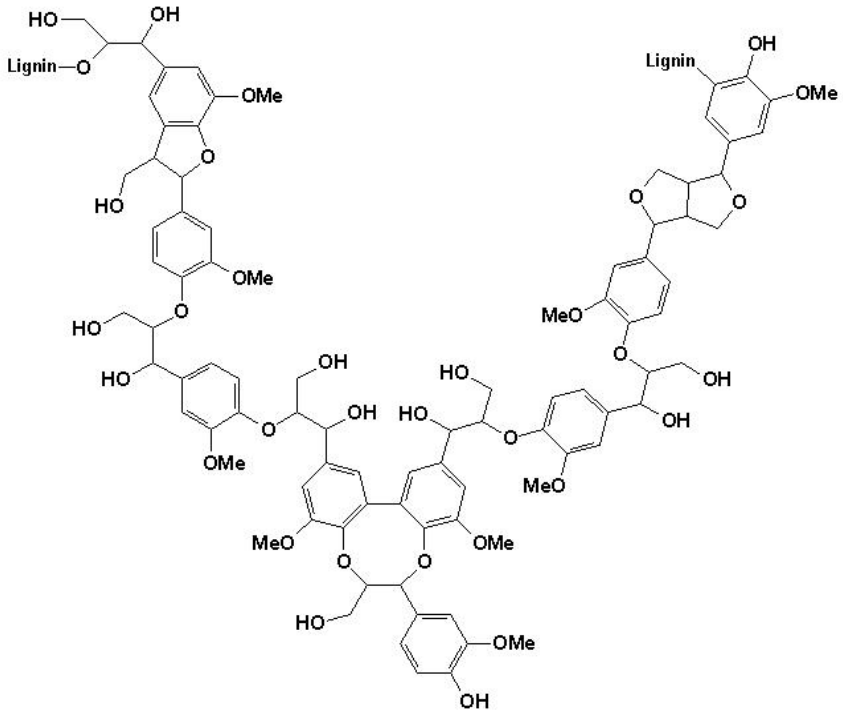


Fig. 2.3.3 Structure of a small piece of the lignin polymer

2.3.2. Cellulases

Cellulase excreting microorganisms such as *Trichoderma reesei* play an important role in nature. By decomposing lignocellulosic material they are a key link in the carbon cycle of the earth. Complete cellulose systems are produced by various microorganisms, such as bacteria and fungi. The cellulolytic bacteria include aerobic species like *Pseudomonads* and *Actinomycetes* and strict anaerobic species such as *Clostridium*. The production of cellulases among fungi is more widespread and includes a wide variety of species. Only some of them are *Trichoderma, Penicillium* and *Aspergillus* (Szengyel, 2000, Persson 1991).

Fungal Cellulases

The term “cellulase” is only a word for a mixture of different enzymes digesting cellulosic material. As shown above the structure of cellulosic material is difficult to catabolise. Many different substances have to be disrupted. Therefore a lot of different enzymes are necessary. Cellulases are not all the same. Their properties and their composition vary depending on their origin. The EC number for the group of cellulase enzymes is the EC 3.2.1.4. Cellulase consists at least of three different enzymes which work synergistically (fig. 2.3.4).

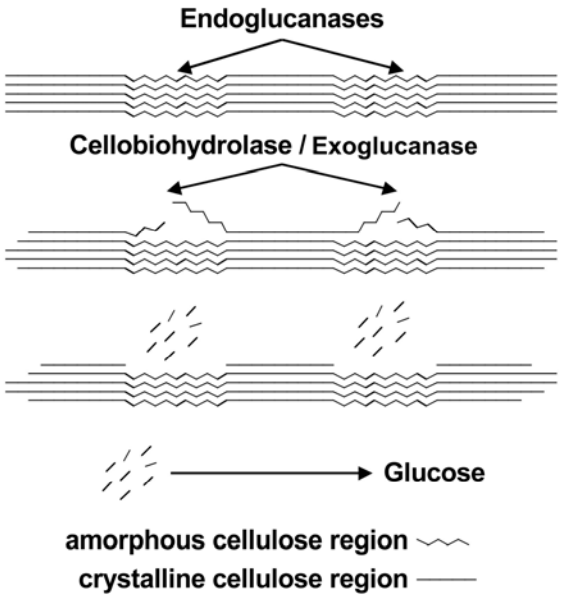


Fig. 2.3.4 Synergistic working of the different cellulose enzymes during the cellulose hydrolysis (SZENGYEL, 2000)

The Endo-glucanase (EC 3.3.1.4) breaks internal bonds in the cellulose chain randomly in the middle of the cellulose chain to disrupt the crystalline structure of cellulose and to expose individual cellulose polysaccharide chains. The Exo-glucanase or Cellobiohydrolase (EC 3.2.1.91) disrupts two glucose units from the end of the exposed cellulose chains which were produced by the Endo-glucanases. The result of this decomposition is the disaccharide cellobiose which is built of two β-1,4-bonded molecules of glucose. *Trichoderma* cultures, which are often used for cellulose hydrolysis, produce two different kinds of cellobiohydrolases (CBHI and CBHII; fig. 2.3.5). One of them (CBHI) works from the reducing end of the cellulose chain and the other type (CBHII) works from the non-reducing end of cellulose chain. The third enzyme that is found in the "cellulase"-complex is the Cellobiase or β-glucosidase (EC 3.2.1.21). This enzyme cuts the liberated water soluble cellobiose molecules into two glucose monosaccharide molecules.

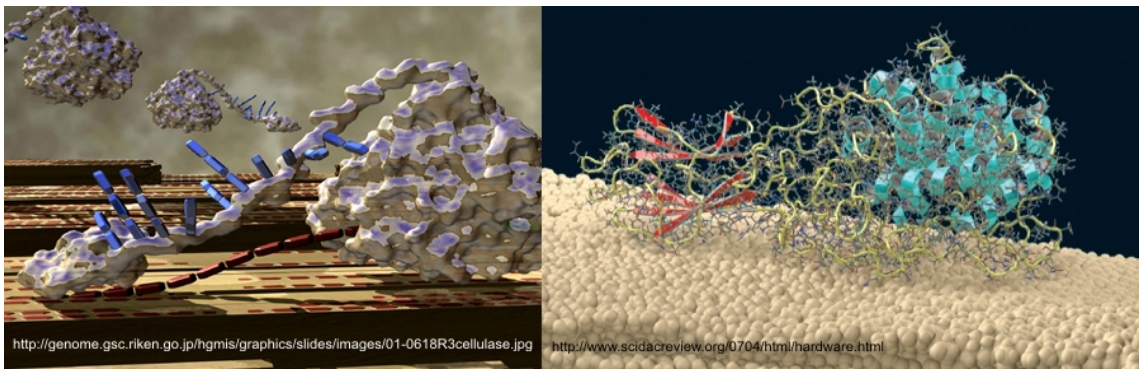


Fig. 2.3.5 Models of cellobiohydrolases

The properties of cellulase enzyme complexes

Most cellulase complexes are effective between pH 3 and pH 7 because of the different pH optima of their single enzymes. Generally the pH optimum for cellulose hydrolysis lies between 4 and 5. The most commonly used cellulase enzyme complexes operate at optimum temperatures between 40 and 50°C and their activity can completely be destroyed after 10-15 minutes at 80°C.

In most processes the high concentration of cellulase enzymes and the long incubation times, which are needed to get a complete hydrolysis of the cellulosic material to usable sugars, produce high costs (GREGG and SADDLER, 1996; SHEEHAN and HIMMEL, 1999). In batch hydrolysis the hydrolysis of cellulose is generally characterized by an initial logarithmic phase associated with the rapid

release of soluble sugars (Fan, 1987). This first phase is followed by a declining rate of sugar production as the hydrolysis proceeds. There are a number of possible explanations for the decreasing of the hydrolysis rate.

One possibility is that as the hydrolysis proceeds, the harder degradable cellulose (the crystalline cellulose) enriches, because the amorphous cellulose is already hydrolyzed.

A second possibility for the declining enzyme's hydrolysis activity is the adsorptive loss of enzymes to the lignin of the biomass. KRISTEN et al. (2006) have worked on solving that problem by using different surface active additives. By adding non-ionic surfactants or polyethylene glycol to the enzymatic hydrolysis of various lignocellulosic substrates it was possible to increase the conversion of cellulose into soluble, fermentable sugars up to 70%. Due to hydrophobic interaction between the surfactants and lignin a prevention of an unspecific adsorption of the enzymes on the substrate's lignin could be observed. This happened because of steric repulsion of the enzymes from the lignin surface.

A third cellulase activity decreasing factor is the inhibition of the cellulase enzymes. End-product inhibition of cellulases is well known. Cellobiose as dominant product of cellulose hydrolysis produces a noncompetitive inhibition of the cellulase complex (Holtzapple et al., 1990). Additionally, cellobiose is unusable for most organisms and in most cellulase systems cellobiose is thought to be a stronger inhibitor than glucose. So cellobiose has to be hydrolyzed to glucose by cellobiase and β-glucosidase respectively. This reaction is competitive or mixed inhibited by its end-product glucose. But nevertheless as a highly fermentable product, glucose is less inhibitory to the cellulase enzyme complex than cellobiose. Because cellobiase is a very rare component in natural enzyme complexes, for successful biomass hydrolysis it has to be ensured that the cellobiase activity is high enough so that all resulting cellobiose is hydrolysed to glucose.

There are several methods to overcome the inhibition of the cellulase complex. One is to increase the concentration of the cellulase. But this strategy with the usage of very high enzyme concentrations increases the cost of the hydrolysis process. Considering the high reqiurement of cellulases for a complete hydolysis, this strategy does not seem to be the right way to solve the problem of enzyme inhibition. Another strategy to solve the inhibition problem is to eliminate the

sugars from the hydrolysate by ultrafiltration (ISHIHARA et al., 1991; TAN et al., 1986). This strategy requires a high degree of technology and energy, and therefore, it does not seem to be the best option for overcoming the inhibition. A third possible way to solve the inhibition problem is to process the hydrolysis of the biomass in a so called simultaneous saccharification and fermentation (SSF). With this process the biomass is hydrolysed with cellulase at the same time as the produced fermentable sugars are fermented by the yeast. The SSF makes it possible to improve the enzymatic hydrolysis. During the process glucose is removed because it is converted to ethanol as soon as the glucose is liberated. So no sugars are accumulated and no end-product inhibition occurs. Additionally, the SSF reduces the costs by eliminating expensive reactions and separation equipment. The only problems that occur using the SSF are the slower hydrolysis of the biomass, the inhibition of the hydrolysis process by ethanol and an incomplete hydrolysis of the substrate. The slower hydrolysis of the biomass occurs because of the lower temperatures at which the fermentation has to be operated. As temperature sensitive microorganisms, yeasts have an optimum temperature of ca. 30-32°C. By contrast, as mentioned above, the optimum temperature for the cellulase enzymes is higher, namely 40-50°C. Thus, the cellulases operate below their optimum temperature. Another problem using the SSF is that the resulting ethanol from the yeast's fermentation acts as noncompetitive inhibitor for the cellulase complex. Up to an ethanol concentration of 100 g/l the binding constant increases approximately linear with increasing the ethanol concentration. In the case of ethanol the noncompetitive inhibition (GHOSH et al., 1982); WU and LEE, 1997) probably results from a reversible denaturation of the enzyme due to the solvent properties of the ethanol (HOLTZAPPLE et al., 1990). The third impediment using the SSF is its incomplete hydrolysis of the substrate. This is caused by the close association of the yeast cells and the adsorbed enzymes with the residue at the end of the hydrolysis process. Thus, some of the released sugar only produces yeast biomass instead of producing ethanol. Additionally, the close association makes it impossible to recycle the yeast cells for further fermentation processes.

2.3.3. Overview of pre-treatment processes for cellulosic biomass

As mentioned above, cellulose in its natural occurrence in lignocellulosic biomass is very resistant to biological attack (WYMAN, 1996-B). So it is recognized that lignocellulosic substrates require some form of pre-treatment (RAMOS et al., 1992). By pre-treating them, they are made less resistant to biological attack (SZENGYEL, 2000), because the pre-treatment is the process by which the surface area of the feedstock is opened up allowing an effective enzymatic hydrolysis. For pre-treatment of lignocellulosic material different strategies are followed. The different pre-treatment methods for lignocellulosic biomass can be divided into physical and chemical methods.

Physical pre-treatments

The physical pre-treatments can generally be divided into two categories, mechanical and non-mechanical pre-treatment methods (CHIARAMONTI, 2007). The physical forces of the mechanical pre-treatments can subdivide the cellulosic material into fine particles which are more susceptible to further hydrolysis steps. The smaller particles have a larger surface-to-volume ratio, thus the cellulose becomes more accessible to hydrolysis processes. Mechanical grinding of cellulosic biomass causes a reduction in crystallinity and, therefore, enhances the effectiveness of enzymatic hydrolysis. Another physical method for pre-treatment is high pressure steaming. Here the substrate is steamed under pressure at high temperatures. This process disrupts the hemicellulose, whereby the hemicellulose is partially hydrolysed to free sugars and to water-soluble sugar oligomers (SADDLER et al., 1993) and as a result the digestibility of the biomass is enhanced.

Chemical pre-treatments

Chemical pre-treatments have been used extensively for the removal of the lignin surrounding cellulose in biomass and for disrupting its crystalline structure (FAN et al., 1980; ANISH, 2009). For chemical pre-treatment alkaline and acetic substances can be used. Sodium hydroxide (NaOH), for example, can be used as an alkaline solvent. Sodium hydroxide causes swelling of the material, leads to an

increase in the internal surface area, decreases the degree of polymerization, decreases the crystallinity of the cellulose, separates structural linkages between lignin and carbohydrates and disrupts the structure of lignin of the pre-treated biomass (FAN et al., 1980).

Another possibility of using chemical substances for pre-treatment is to use acids. Acids primarily serve as catalysts for the hydrolysis of the cellulose than as reagents for pre-treatment (FAN et al., 1980). The acids which are mostly proposed for pre-treatment are sulphuric acid (H_2SO_4) (MOSIER et al, 2005), hydrochloric acid (HCl) and phosphoric acid (H_3PO_4). In the last decades, a lot of different acid processes have been developed for cellulose pre-treatment and hydrolysis and a lot of research has been done to determine the effect of the acids on the cellulosic biomass.

2.3.4. Today's pre-treatment processes for lignocellulosic biomass

Nowadays a combination of chemical, physical and enzymatical methods is used for successful raw material pre-treatment. Most of the methods used today are still methods at high temperatures (>170°C) using diluted mineral acids or alkali as an additive to remove the hemicellulose and make the material more porous. In a second step the cellulose is digested to fermentable glucose by enzymatic cellulase complexes. Here only processes that are used for pre-treatment for the ethanol production from lignocellulosic biomass are described more precisely. Beside these processes other processes as the "organosolv process" or the "acetosolv process" are used in the pulp and paper industry to produce pulp the cellulosic intermediate at the paper production process. For the organosolv process organic solvents are used to remove the lignin from the biomass. This process is operated at temperatures of ca. 190°C. Instead of this quite high temperature the acetosolv process only needs a temperature of ca. 110°C (at normal pressure). Instead of organic solvents glacial acetic acid is used to liberate the cellulose from the lignocellulosic raw material. During the chemical reaction the ether bondings between cellulose hemicellulose and lignin are broken. The result of that process is a black pulp that has to be bleached for the paper production. Beside these both processes the pulp and paper industry also uses other processes e.g. the "formacell-process" that is similar to the acetosolv process but

uses formic acid and acetic acid as reagents, the “milox process” that is using and hydrogen peroxide and the “alcell process” that works with an ethanol/water mixture. But none of these processes is known to be used for the pre-treatment of lignocellulosic biomass for the production of bio ethanol.

Concentrated acid pre-treatment

In the following only today’s most frequently offered and used processes shall be described. The first process is similar to a very old process and has been known since the middle of the 19th century (FEITLER, 1903). Although the technology is very old, it makes it possible to convert a wide variety of biomass feedstock into sugars. But these day’s new technologies such as modern control methods and new construction materials are used. These newer technologies make it possible to improve the process effectiveness and, thereby, make the process economically viable. The process is offered as a cellulose using process by Arkenol Inc. and the franchisee Blue Fire Ethanol. The process is shown in fig. 2.3.6. First the feedstock is filled into a conical, jacketed reactor made of 316SS and coated with a Kynar®-like coating (Kynar® is an engineered polyvinylidene fluoride thermoplastic material that has the characteristic stability of fluoropolymers to harsh thermal, chemical, and ultraviolet environments) for acid resistance. In this vessel the first hydrolysis and decrystallisation step is performed with sulphuric acid concentrations between 25 and 90% (FARONE et al., 1996; FARONE et al., 1996-II; FARONE et al., 1998) by stirring the mixture with a screw mixer which is mounted on an orbit arm that sweeps the inner walls of the reactor to ensure proper mixing. After pre-treatment the mixture is discharged into the plate or frame filter units. Filtration is used to separate the acid/sugar mixture from the lignin and other solids available from the 1st hydrolysis step. The solids are transported to a second hydrolysis step with acid concentrations between 20-30% sulphuric acid (FARONE et al., 1996; FARONE et al., 1996-II; FARONE et al., 1998). The filtrate, a mixture of acid and C_5 and C_6 sugars, is collected and fed to the chromatographic separation system (FARONE et al., 1996-II). After the second hydrolysis step, it is filtered again to separate the acid/sugar mixture from the resulting lignin. Like to the process after the first hydrolysis step the filtrate (acid/sugar mixture) is collected and fed to the chromatographic separation system

(FARONE et al., 1998). In both filtration processes the overall moisture content is reduced to ca. 50% via a hydraulic piston. A chromatographic separation system is used to recover the acid. In this separation system incoming raffinate streams containing sugar at a 12-15% concentration can be purified to purities of greater than 98%. The sugar liquor obtained is neutralized with lime and centrifuged to remove the resulting gypsum before fermentation. The recovered sulphuric acid is re-concentrated by heating with steam to recycle it for the biomass hydrolysis steps.

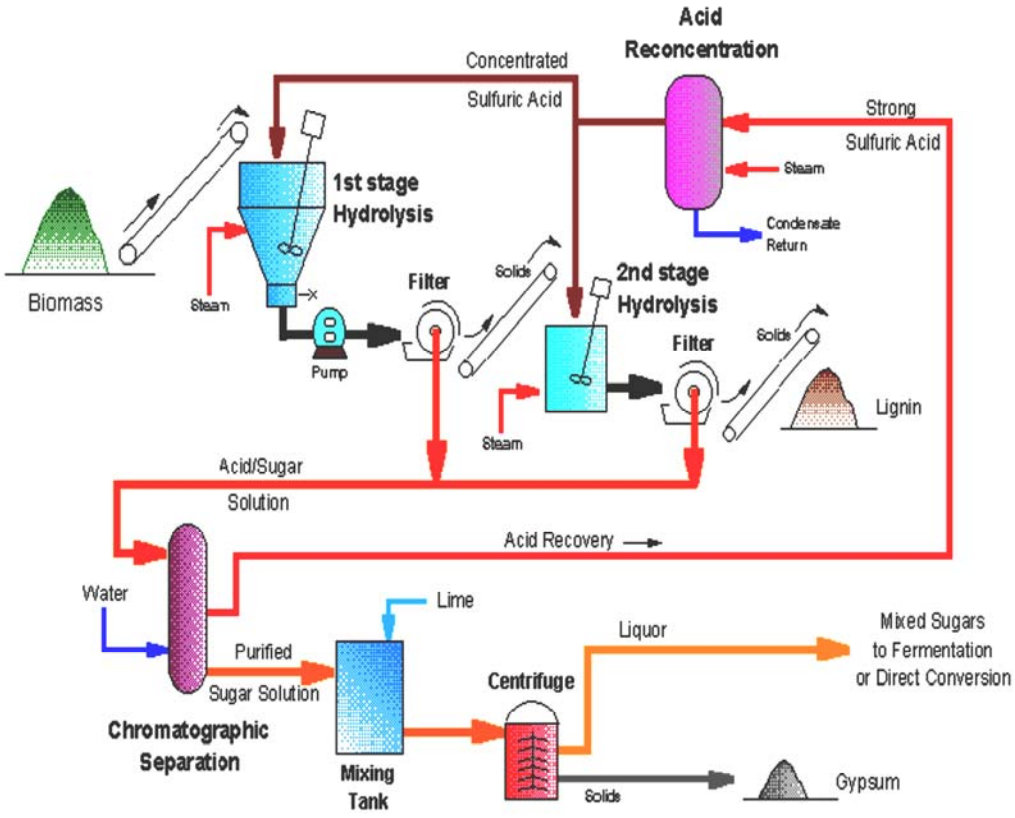


Fig. 2.3.6 Conversion of cellulose and hemicellulose to mixed sugars using Arkenol's concentrated acid hydrolysis process (simplified flow diagram) Source: www.Arkenol.com

Dilute acid hydrolysis

Another patented process is the process of the canadian logen Corporation for ethanol production from lignocellulosic materials such as straw, corn stover and energy crops like grasses.

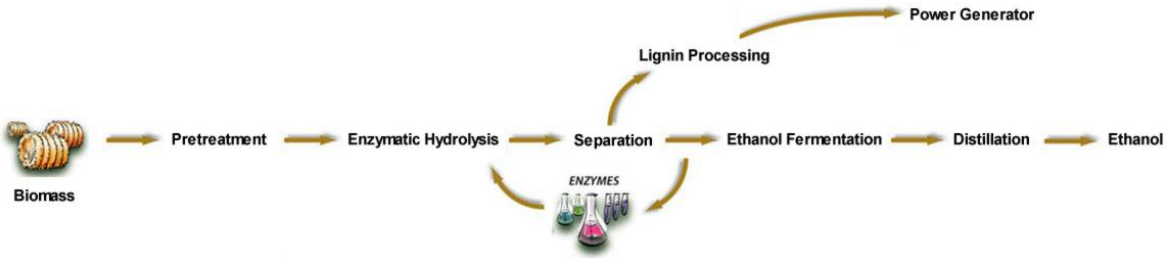


Fig. 2.3.7 Iogen's process for converting lignocellulosics to ethanol

In the Iogen process (fig. 2.3.7) the feedstock in the form of bales is chopped and milled to small particles (FOODY, 2006) by hammer milling, whiley milling, szego milling or a combination thereof (GRIFFIN et al, 2007). In a pre-treatment reactor sulphuric acid is added to reach a concentration of 0.5-1% for hardwood and 5-7% for agricultural fibers respectively (FOODY, 2006; GRIFFIN et al, 2007). Then the mixture is heated to 200-280°C with high pressure steam (FOODY, 2006; FOODY and TOLAN, 2006; FOODY and ANAND, 2006; FOODY and ANAND, 2008). The material is maintained in this state for less than 1min. Then the pressure is released rapidly. The result of this pre-treatment is the destruction of the fibrous structure of the feedstock so that the pre-treated material has a muddy texture, a slightly sweet smell and dark brown colour. In the following enzymatic hydrolysis step the cellulase enzymes convert the cellulose to glucose. The pre-treated feedstock with a total solid concentration of 15-20% is transferred into a slurry tank and is adjusted to a pH of 4.5-5 with caustic soda (FOODY and TOLAN, 2006; FOODY and ANAND, 2006; GRIFFIN et al., 2007). There it is maintained at 50°C and mixed with a self-produced crude cellulase broth (EDWARDS et al., 2009/2). The content of the tank is agitated slightly to move the material and keep it dispersed. The hydrolysis then proceeds for 5-7 days and the viscosity of the slurry decreases (TOLAN 1999 und TOLAN 2002). After the hydrolysis the insoluble particles (mostly lignin) are separated from the sugar rich part of the hydrolysis liquor by a frame or plate filter (TOLAN 1999 und TOLAN 2002). A small part of this liquor is used as a feedstock to produce the enzymes. The other glucose, xylose and arabinose rich solution is pumped to fermentation tanks and fermented to ethanol (EDWARDS et al., 2009/1). The solid lignin cake resulting from the filtration is spray-dried to a moisture content of <10% and burned in a solid-fuel boiler to generate power and steam to run the plant (TOLAN 1999 und TOLAN 2002).

A third process, which was used at the University of Lund and today also is patented by the company SEKAB, is the usage of sulfite as impregnation agent for corn stover and wood (ÖHGREN, 2005; ÖHGREN, 2006; SZENGYEL, 2000). This kind of process is well known from the sulfite pulping process which is also used in the paper industry. In this process (fig. 2.3.8) first the cellulosic material is dried, milled and sieved and the fraction between 2 and 10mm is used for laboratory tests at the university Lund. In the ethanol plant the raw material is screened to remove oversize material.

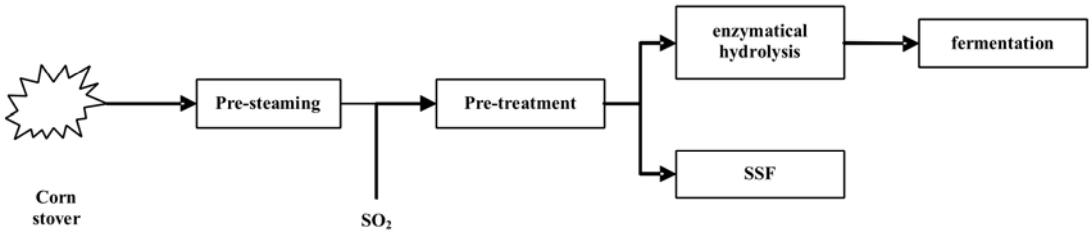


Fig. 2.3.8 Lund's pre-treatment process for the ethanol production from corn stover

The material is impregnated with SO_2 to an uptake of up to 3% of SO_2. Then the material is pre-treated in a pressure reactor at temperatures of 175-240°C for 2-10min (ÖHGREN, 2005; ÖHGREN, 2006; VAN DER MEULEN et al., 2008). In the ethanol plant of SEKAB, the material alternatively also can be pre-saccharified with other acids, at temperatures of 170-200°C, to leach out various types of sugars, especially the hemicellulose components Xylose, Arabinose, Galactose or Mannose. After pre-treatment the pH has to be adjusted to 5.0 with alkaline to make the performance of a simultaneous saccharification and fermentation process (SSF) or a separate enzymatical hydrolysis and fermentation process (SHF) possible. Alternatively to the enzymatic digestion of the lignocellulosic biomass, at the SEKAB ethanol plant the cellulose hydrolysis also can be performed using diluted acid at a temperature of 200-300°C. Before the yeast is added to the process slurry, the process slurry is separated to a clear liquid solution and a lignin solid phase in a membrane filter press. After detoxification of the sugar solution, essential nutrients have to be added to enable the yeast to grow and ferment the slurry to ethanol. To improve the fermentability of the yeast first it can be grown as batch cultivation on media at ideal conditions and later on as fed-batch culture. In the fed-batch phase hydrolysate from the pre-treatment containing inhibitors is added. This step is done to allow the yeast to acclimate to the worse conditions of the hydrolysate slurry. When the fermentation is finished

the yeast is separated in a decanter or separator from the ethanolic solution that is distilled to gain the endproduct (ethanol). The remaining stillage is evaporated to increase the dry substance. Alternatively the stillage can be used to produce biogas. A flowchart of the SEKAB process can be found at www.sekan.se.

Autohydrolysis process

The energy company Dong from Denmark currently is erecting a demonstration plant that operates using a process (fig. 2.3.9.) which is comparable to the processes shown above. For realising the project Dong founded a company called Inbicon. Similar to the Iogen process the biomass is reduced in size to 26-70mm. During the pre-treatment apart from chemical additives like acid or SO_2, only hot water is used at 110-250°C (preferably 180-200°C) for 1-60min (preferably 5-15min). After the pre-treatment the lignocellulosic biomass at least has to contain a dry matter of 20%. Then lignocellulytic enzymes (e.g. Novozym 188, and Celluclast 1.5 FG L) are added and the biomass is hydrolysed in a special hydrolysis reactor that works on the freefall mixing concept similar to a drum or a tumble mixer. A homogenous mixing of the biomass and the enzymes as well as a further size reduction is realised by the addition of steel balls into the reactor and an alternated rotating direction of the vessel.

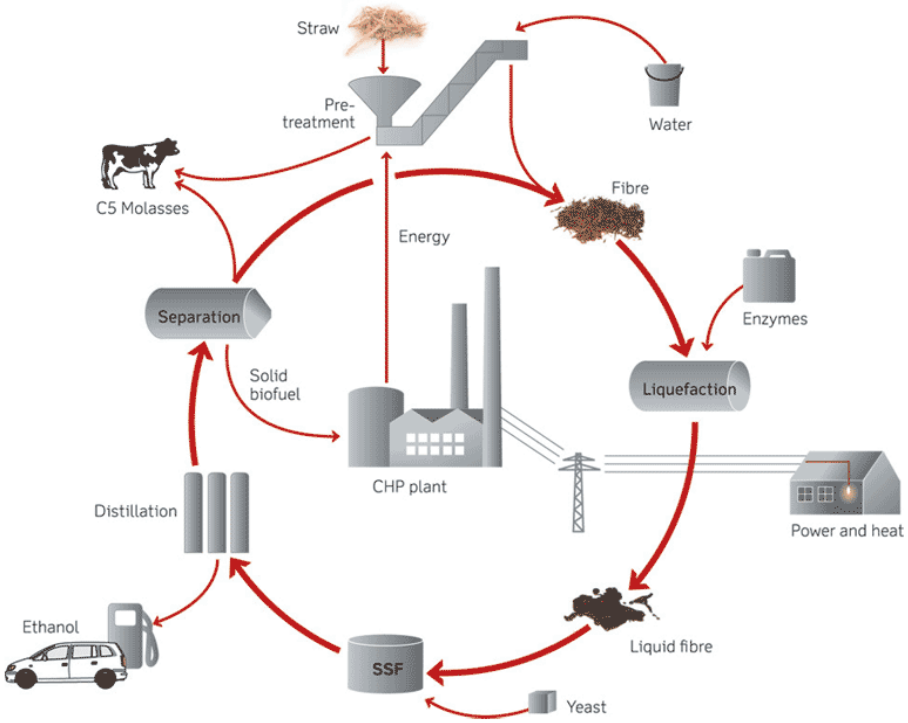


Fig. 2.3.9 Inbicon biomass to ethanol process

After up to 72 hours of enzymatic treatment with up to 10 FPU/g dry substance the biomass is transferred into a fermenter where it is mixed with yeast and fermented to ethanol in an SSF process. After complete fermentation the slurry is distilled to separate the ethanol from the water and the solids. The residual stillage then is separated to a C5 molasses, which can be used as animal feed, and the solid fibres. The special idea of Inbicon is to build such biomass to ethanol plant near an existing power plant and use the surplus heat from the power plant for the process. Using this process a cellulose hydrolysis between 30-50% can be realised (FELBY et al., 2009).

2.3.5. Inhibitors built during the pre-treatment processes

Regarding all the described pre-treatment processes using additives, it is important to note that in all such processes the formation of degradation products of liberated sugars from hemicellulose and cellulose and lignin respectively can occur due to the high temperatures and the acid conditions used (LARSON et al, 1999). These degradation products can exert inhibition in various steps of the further process (BANERJEE, 1981; NAVARRO, 1994; PALMQVIST and HAHN-HÄGERDAL, 2000; TAHERZADEH, 1999; TAHERZADEH, 2000; HORVÁTH et al, 2003; CHUNG and LEE, 2004). The inhibitory compounds can be divided into three major groups according to their chemical nature. The first group are the furan derivates 2-furanaldehyde (furfural) and 5-hydroxymethyl fufural (HMF). Furfural is formed when pentoses such as xylose or arabinose are thermo-chemically broken down. Fig. 2.3.10 shows the reaction mechanism of the degradation of xylose to furfural.

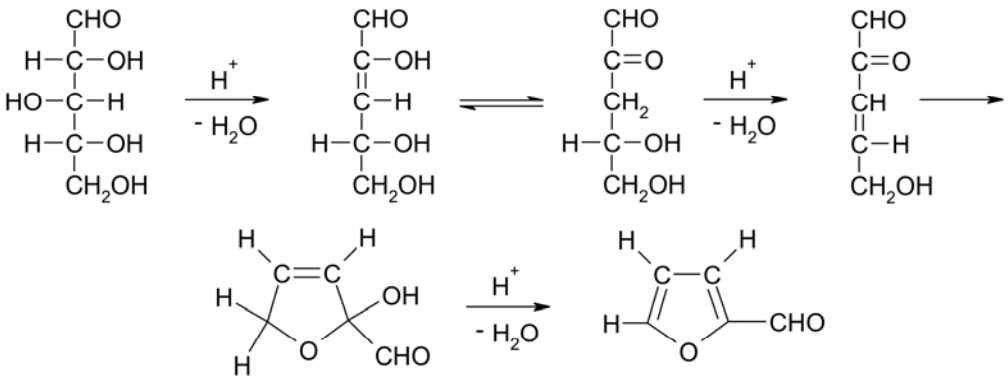


Fig. 2.3.10 The degradation of xylose to 2-furanaldeyde (furfural)

Like the pentoses degradation reaction mechanism, hexoses such as glucose, a degradation product of cellulose, can also be broken down. When that occurs another furfural, the hydroxymethylfurfural and 5-hydroxymethylfurfural respectively, is formed.

The second group of inhibitors which appear after pre-treatment are short chain aliphatic acids. Typically, hemicellulose hydrolysates contain various amounts of acetic acid and formic acid. The acetic acid is simply formed by the deacetylation of the hemicellulose (SZENGYEL, 2000; GARROTE et al, 2001). The formic acid is built as a further degradation product of HMF and furfural.

The third group of inhibitory components includes various phenolic substances, that are liberated by a slight lignin degradation.

2.4. Strategies for the enzymatic hydrolyis and fermentation of glucane-containing raw materials to ethanol

To transform glucane-containing biomass like starchy materials and lignocellulosic biomass, respectively, to ethanol different strategies can be used. One strategy is to hydrolyse the raw material in a first step and afterwards ferment the resulted sugar solution to ethanol in a second step. As already mentioned above, some enzymes can be inhibited by higher concentrations of their products. If this happens no further glucane hydrolysis to fermentable sugars can be realised. But another strategy can help to overcome this problem. Instead of performing the process in two steps the complete hydrolysis and fermentation is performed in one step. Such processes are called SSF-processes (simultaneous saccharification and fermentation processes). In this chapter the differences of both strategies will be described.

2.4.1. The separate hydrolysis and fermentation process (SHF)

In normal distillery processes the material which is to be converted to ethanol is processed in two different steps. In the first step the material is pre-treated to hydrolyse the polysaccharides and digest them to fermentable sugars. In the following second step the resulting sweet slurry is fermented to the end-product ethanol by yeast cells of the species *Saccharomyces cerevisiae*.

The hydrolysis of starch-containing materials

Starch-containing material, such as wheat, is first milled to break up the cereal grain and reduce the particle size in order to facilitate subsequent penetration of water into the material. Then the milled cereal is mashed with starch digesting amylolytical enzymes. In order to make the starch accessible for enzymes the granular structure of the starch must be broken down first. This process is known as gelatinization. In this process the starch granules start to absorb water and swell. Thereby they lose their crystalline structure until they become large gel-filled sacks which tend to fill all the available space and break with agitation and abrasion. The starch is then accessible for enzymes attack. The technical enzyme α-amylase, which is mostly produced from fungi, randomly digests the α-1,4-linkages of the starch. The resulting shorter oligosaccharide strains, which are called dextrins, produce a lower viscosity of the mash. To obtain fermentable sugars, the slurry has to be liquefied with other enzymes. Here for that part, technical enzymes such as glucoamlyase, is used to digest the dextrins to easily fermentable glucose.

After complete saccharification of the cereals the mash is cooled to ca. 30°C and is now ready for the yeasts fermentation. This transformation of sugar to ethanol is then performed in a second step in a fermentation tank.

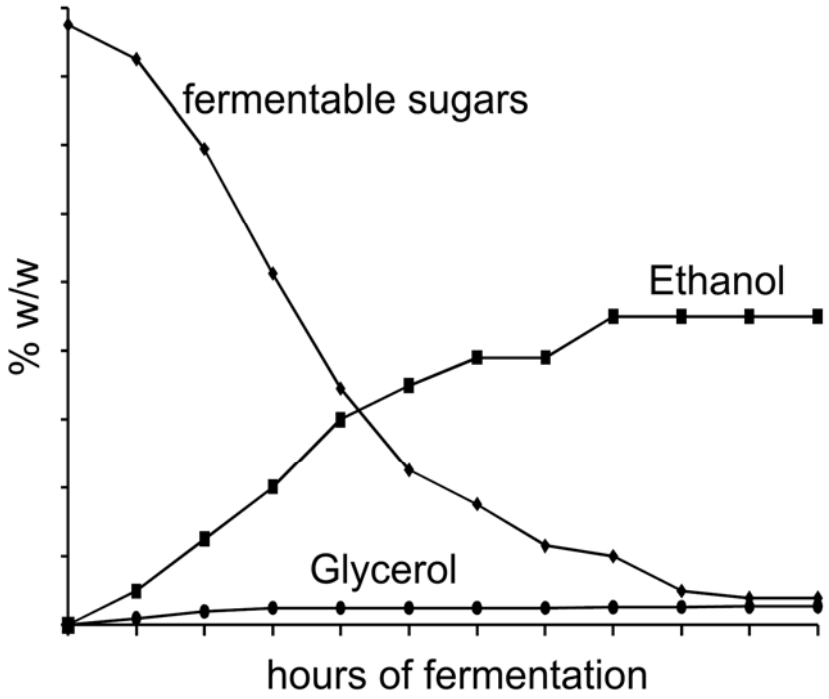


Fig. 2.4.1 Progress of a classical fermentation process

The progress of the fermentation is shown in fig. 2.4.1. At the beginning of the fermentation a huge amount of fermentable sugars can be found. With increasing fermentation time the concentration of sugars decreases while the ethanol is formed. During the first hours of fermentation some glycerol is produced. But after some time the level of glycerol remains the same. The longer the fermentation lasts, the more the ethanol concentration increases until all the fermentable sugars are converted to ethanol.

The hydrolysis of cellulosic biomass

As with the process for starchy materials, in a first step the biomass is pre-treated with different processes as already shown in chapter 2.3.3 to make the material accessible for the following enzymatic attack. As mentioned before in chapter 2.3.2, cellulase enzyme complexes are used to digest the structure of the pre-treated biomass. In a second step, that is similar to the process described above the resulting fermentable sugars are transformed to ethanol by yeast cells during the following fermentation process. The progress of the fermentation is the same as the progress of the starchy material.

2.4.2. The simultaneous saccharification and fermentation process (SSF)

The SSF process differs from the SHF processes which has been described before. As already mentioned in chapter “The properties of cellulase enzyme complexes”, the pre-treated material is digested by the enzymes to fermentable sugars parallel to their fermentation to ethanol. No separate hydrolysis step is used. Unlike the SHF process, the detectable concentration of fermentable sugars is much lower during this process because the fermentable sugars are primarily produced during the fermentation. An important advantage of the SSF process is the fact that the inhibition of end-products, as in the case with glucose and cellobiose inhibition of cellulases, is reduced. This happens because the sugars are fermented by the yeast cells as soon as they appear in the mash. An SSF processes can be used for all kinds of biomass, in particular for processes where enzymes are inhibited by end-products. Fig. 2.4.2 shows the typical course of a

normal SSF process. The soluble sugars first accumulate during the initial phase, because they are produced faster than they are fermented by the yeast cells. During this phase the yeast cells eventually multiply and afterwards ferment faster, while the rate of enzymatic hydrolysis decreases. Then the SSF continues at a low concentration of soluble fermentable sugars, while the concentration of ethanol still increases. When using an SSF process, the production of fermentable sugars has to be high enough to ensure the yeast does not die. Such a situation can occur when an SSF is performed at high temperatures and is forced to yield in high ethanol concentrations (GROHMANN, 1993).

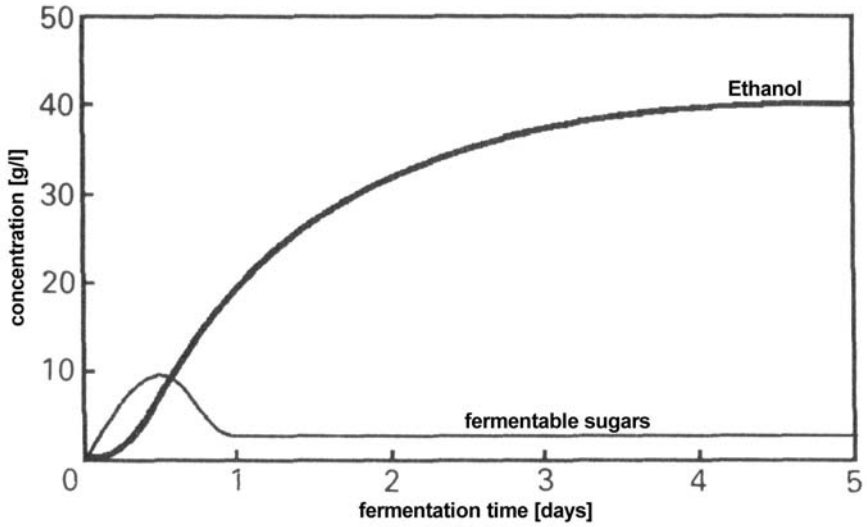


Fig. 2.4.2 Course of a typical simultaneous saccharification and fermentation process (SSF), source: Grohmann (1993) figure modified

In the case of the SSF process using cellulosic biomass as pre-treated raw material the concentration of cellulose as the potential usable substrate is important, because its concentration determines the possible final ethanol concentration. Most of the cellulosic substrates are quite unique, because they are high in fibrous and bulky irregular particles respectively, which do not dissolve during the complete biomass hydrolysis of the SSF process. These solid particles are difficult to stir at concentrations exceeding 8-12%mas (GROHMANN, 1993). MOHAGHEGHI et al. (1992) examined the influence of the dry substance content of pre-treated wheat straw (7.5-20%) and showed that the optimal concentration for cellulose was ≤ 15%mas (≤ 24.4%mas straw dry substance concentration). However one problem that seems to occur under such conditions is that the ethanol tolerance of the yeasts decreases (GROMANN, 1993).

2.5. Distillation

Once the fermentation has finished, the mash is distilled. Here the ethanol is separated from the rest of the mash by boiling the mixture in a distillation column, where the ethanol, some water and other volatile substances are evaporated. The term distillation is based on the Latin word “destillare” = dripping down, which therefore provided the name for the process. By distilling an alcoholic mash an alcohol enriched distillate is obtained. The concentration of the resulting ethanol depends on the realization of the distilling process and the construction of the distilling column. The residue of a distillation process other than the ethanol is called the stillage.

2.5.1. The basics of distillation

Although the boiling points of water (100°C) and ethanol (78.39°C) differ strongly, it is impossible to get an accurate separation of ethanol and water by distillation. The mixture of these two liquids will boil at any temperature between 78.39°C and 100°C, depending on the ratio of alcohol to water. However, to understand how the distillation manages to separate the ethanol from the rest, it is necessary to look at how distillation works.

The typical course of the distillation of a two-component mixture such as ethanol/water, is shown in fig. 2.5.1. It is the vapour-liquid equilibrium diagram for the ethanol-water system at atmospheric pressure. The diagram shows the mole percent concentration of ethanol in the liquid (X axis) versus the mole percent concentration of ethanol in the vapour (Y axis). The fact that the heat or energy required to vaporise or condensate a mole of ethanol is approximately the same as the heat or energy required to vaporise or condense water or any mixture of both makes it possible to analyse the relationship of water to ethanol in a distillation process graphically (KATZEN, 1999). The 45° line in the vapour/liquid equilibrium diagram represents the points at which the concentrations of ethanol in the vapour and in the liquid are equal. The curved line is the equilibrium curve. This curve describes the composition of the concentrations of ethanol in the liquid and the vapour in the equilibrium status at a constant temperature and pressure. So the equilibrium is a status at which there is no further interchange in the

liquid/vapour ratio or in the alcohol/water ratio within either the liquid or vapour mixture. As can be seen in fig. 2.5.1, the ratio of alcohol to water in the vapour phase is generally greater than the ratio in the liquid phase. This is, because the alcohol is more volatile than water. This is the main characteristic of a liquid-versus-vapour state of an alcohol/water mixture that permits us to distillate off an increasing concentration of alcohol.

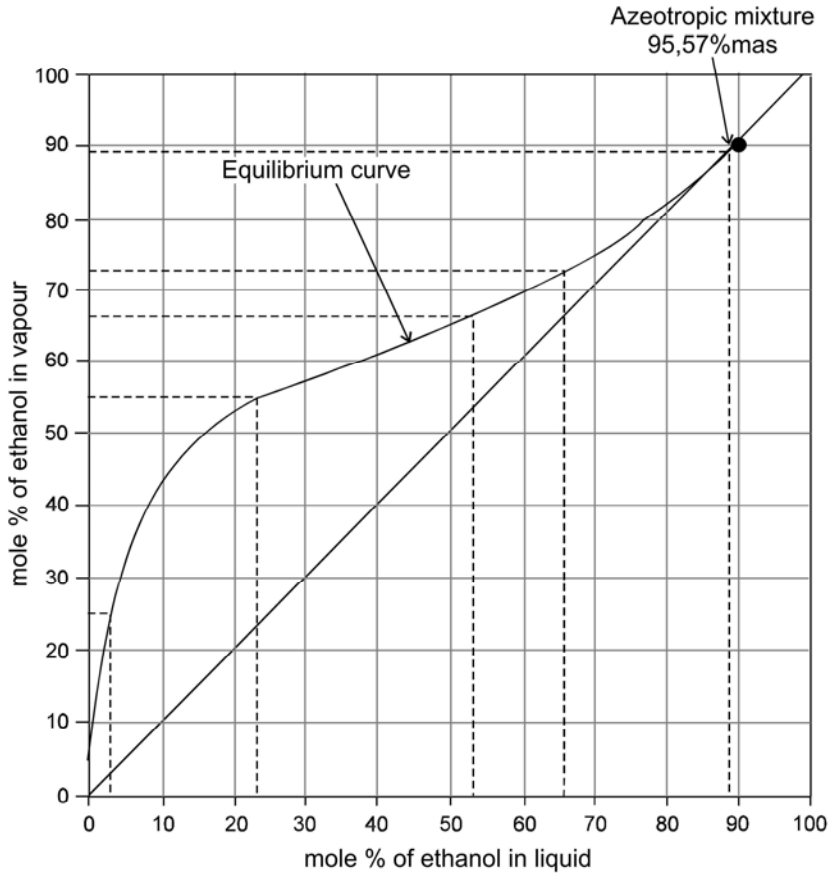


Fig. 2.5.1 Equilibrium diagram of an ethanol/water system at atmospheric pressure.

2.5.2. Continuous distillation

Howerver in order to produce ethanol with a higher concentration, the enrichment effect of a single distillation step has to take place many more times. This can be achieved by distilling the resulting distillate in a simple follow-up distillation step or to distillate the mash in a distillation column. Here 13 to 15 pot stills are stacked to build one column. Fig. 2.5.2 shows the arrangement of such a distillation column. Here the different trays and plates respectively or bubble cap trays produce the effect of different distillation vessels. On every single bubble cap tray the

distillation process as described above occurs. Because of the enrichment of the ethanol in the vapour and the distillate respectively, the heated mash on every single bubble cap tray gets poorer regarding its ethanol concentration. Regarding the complete column, by bringing about a controlled series of successive sequences of evaporation, condensation, re-evaporation and re-condensation, each re-condensation from the previous vapour state leads to a higher alcohol concentration.

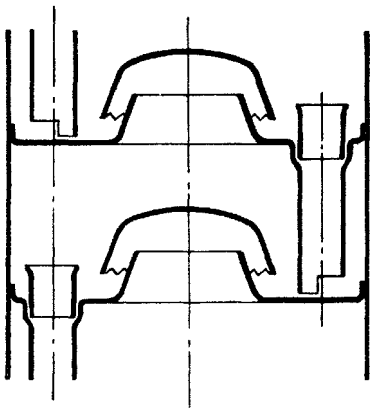


Fig. 2.5.2 Arrangement of the bubble cap trays for a continuous distillation column

Table 2.5.1 shows an example for the enhancement of ethanol for a repeated mash distillation of a mash containing 10%vol. The number of trays needed for a distillation column and therefore, the height of the column depend on the final ethanol concentration to be attained by the distillation process. However, the maximum concentration of ethanol that can be achieved by distilling is 95.57%mas and 97.17%vol respectively. At this concentration, the two substances ethanol and water cannot be separated further, because the mixture forms a so-called “azeotropic mixture”. An azeotropic mixture is a solution of two components that form a vapour with the same concentration as the solution. Therefore, no further enrichment can be achieved in the more volatile component (also shown in fig. 2.5.1).

The column for a continuous distillation can be divided into two sections. The first section, which is located in the lower portion of the column, is the stripping section, while the upper portion of the column, where the ethanol/water mixture already has a higher ethanol concentration, is the rectifying section. For distilling the ethanol up to a higher concentration the process of a continuous distillation

involves a controlled flow of preferably preheated mash, which is fed into the top of the stripping portion of the column. The poured in liquid alcohol-water mixture trickles downwards through the column. Its flow is impeded by a series of plates. A mixture of water vapour and alcohol vapour rises up from the bottom of the column. The ascending vapour and the trickling mash are mixed together on every single plate and, as a result the mash is heated. This heating of the mash allows the alcohol to evaporate and the alcohol/water mixture to enrich in ethanol concentration. At any given point along the column, there is more alcohol in the vapour than in the liquid, but not as much as there should be according to the equilibrium principle. Since the alcohol concentration in the vapour has not reached equilibrium, its vapour pressure causes it to evaporate out of the liquid, and water condenses out of the vapour. These two processes take place simultaneously, because the first (the vaporization) requires the heat and the second (the condensation) produces the heat. During the distillation process about the same amount of alcohol evaporates as the amount of water that is condensed. Thus, the vapour which is moving up the column constantly increases in its alcohol content. Whereas the condensed liquid which is trickling down the column constantly loses alcohol. Hence, the highest alcohol concentration in both liquid and vapour is achieved at the top of the column. And logically for a complete distillation no alcohol can be found at the bottom of the column.

	Alcohol content [%vol.]
Mash	10,0
1st distillate	32,7
2nd distillate	58,3
3rd distillate	74,8
4th distillate	83,2
5th distillate	87,3
etc.	

Table 2.5.1 Enhancement of ethanol for a repeated distillation

In a continuous operation, the column is brought to a balanced-operation state. In this state a continuous feed input of mash, a continuous outflow of stillage, a continuous input of steam and an output of highly concentrated ethanol vapour has to be managed. The ethanol rich vapour is condensed in a dephlegmator or reflux condenser, where the ethanol concentration of the vapour phase is enriched

further. This further enrichment of the ethanol/water vapour produces a reflux of ethanol which is condensed in the dephlegmator. The large fraction of refluxed and thereby recirculated ethanol falls down into the top of the column and so controls the final concentration of the yielded ethanol. Without the reflux there would be no liquid in the rectifying section of the column, which means that no separation would then occur in the rectifying section.

If the vapour composition at every point of the column is plotted versus the corresponding composition of the liquid, the result does not look like the equilibrium diagram shown in the diagram of fig. 2.5.1. Fig. 2.5.3 shows the result of the distillation.

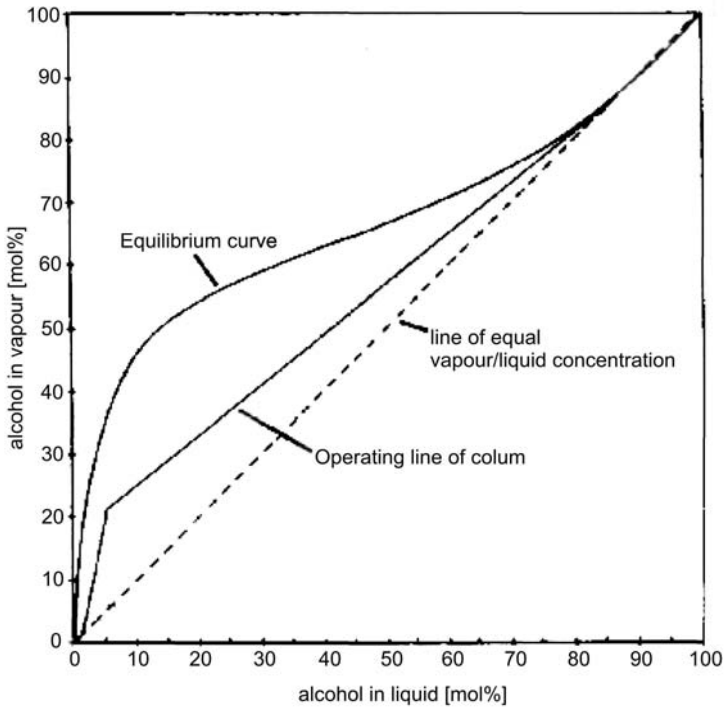


Fig. 2.5.3 The operating line of a distillation with reflux

The "operating line" of the diagram is higher than the line of equal concentrations because of reflux which trickles down the column was part of the vapour. In the equilibrium diagram shown in fig. 2.5.1 all vapour would be condensed to liquid on the next tray with an enriched ethanol concentration. But here some ethanol off the vapour is condensed and falls down on the same tray. The greater the reflux is, the smaller the amount of product that is obtained. However the ethanol concentration is higher.

2.5.3. Azeotropic distillation

As mentioned above, a normal distillation produces an ethanol concentration with maximum 95.57%mas and 97.17%vol, respectively. But if ethanol is blended with gasoline, the fuel grade ethanol has to be almost completely dry (BIBB SWAIN, 2003). One possible way to remove the residual water from the ethanol is the so-called azeotropic distillation method. Here the azeotrope is broken by adding another component which generates a new lower-boiling azeotrope that is heterogeneous. Normal distillation techniques of a rectification column are used for processing an azeotropic distillation of an ethanol/water mixture. By addition of cyclohexane, in former times benzene was used, a ternary azeotrope with lower boiling point can be formed. When this mixture is boiled in a rectification column, on every tray the ternary azeotrope vaporizes leaving a residue composed almost entirely of the excess ethanol. During such a rectification, nearly absolute ethanol remains in the lower region of the column. The ternary mixture including the water is collected at the top of the column. Outside of the column when this mixture is condensed, it easily can be separated in a decanter into two phases. One phase which is rich in cyclohexane directly is recycled into the rectification column. The second phase which is poor in cyclohexane contains most of the water as well as the condensed ethanol. For separating the cyclohexane this mixture is distilled in a further column. The gas phase leaving the column at the top is recycled to the decanter. An ethanol/water mixture containing no cyclohexane remains at the bottom of this column. This mixture is recycled into the first distillation steps. Using such a process, an ethanol concentration of approx. 99.8%vol. can be yielded (KREIPE, 1981).

2.5.4. Molecular sieve

Another possible way to separate the ethanol from the water in an azeotrope is to use a molecular sieve. Before the ethanol is dried in the molecular sieve, it has to be rectified. Today, molecular sieves consist of synthetic zeolites with a crystalline lattice structure that contains openings (pores) of a precise size (fig. 2.5.4). The size of these pores is usually measured in Å. For the dehydration of ethanol mostly a zeolite type of 3 Å is used (BIBB SWAIN, 2003). This is because the pores of the

zeolite have a pore size of 3 Å in diameter, while water molecules are 2.8 Å and ethanol molecules are 4,4Å. Therefore, the water molecules are strongly attracted into the pores while the ethanol molecules are excluded. The most modern molecular sieve dehydrators use a process that is called a “pressure swing adsorption”. The term “pressure swing” refers to the fact that the dehydrator uses high pressure for removing water from the feed stream and low pressure for regeneration of the molecular sieve (removing of water from the zeolites). Using molecular sieves it is possible to produce dried ethanol of concentrations up to 99.9%vol.

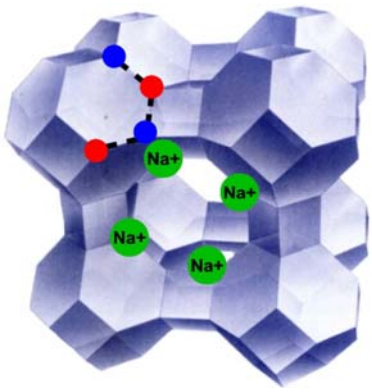


Fig. 2.5.4 Zeolite of a molecular sieve type A pore size 4Å source:http://www.gracedavison.com/eusilica/Adsorbents/product/zeolite_mo)

Fig. 2.5.4 shows a zeolite particle of a molecular sieve. Here a type A particle with sodium as counter-balance cations is shown. If the sodium atoms of the zeolite are exchanged with larger potassium ions, the pore opening of the zeolite is reduced to approximately 3 Å and a type 3 zeolite is generated. In addition to the higher ethanol concentration yielded, the molecular sieve has further advantages. Firstly azeotropic distillation is the more difficult operation with the higher capital costs. Secondly, molecular sieves do not require the use of carcinogen substances and, thereby, the plant workers do not have to work with such substances.

2.6. Production of biogas or bio-methane

Biogas or rather bio-methane is a mixture of mainly methane and carbon dioxide that is formed when organic material is anaerobically digested and fermented by bacteria. The product with the higher value is methane which can be used energetically. Beside the two already mentioned main components minor

components including water vapour, hydrogen sulphide, ammonia, hydrogen, nitrogen, lower fatty acids and alcohols also appear. However to describe how bio-methane is produced, it is necessary to take a closer look at the process.

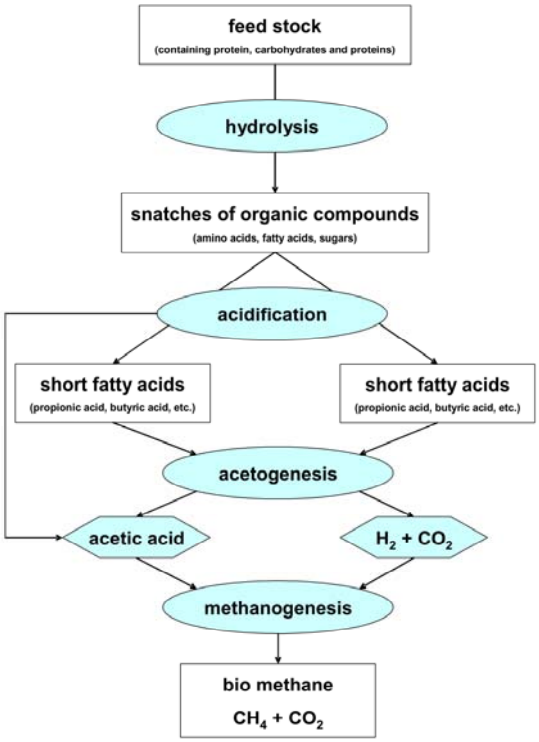


Fig. 2.6.1 Schematical description of the anaerobic digestion of biomass to methane

To produce bio-methane, different anaerobic microorganisms have to participate. These different microorganisms, whose relation of quantity is influenced by the starting substrate, all have different pH- and temperature optima and, additionally, different digestion and fermentation speeds in the process.

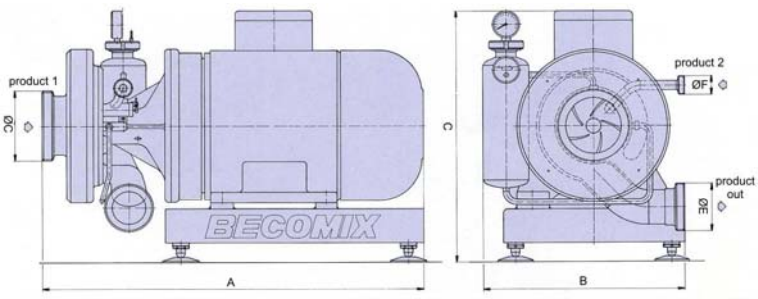
Today the bio-methane process is divided into four parallel working and meshing biochemical processes that allow the anaerobic digestion of organic biomass to methane. Fig. 2.6.1 shows the flow chart of such an anaerobic digestion process. In the first step, which is called the “hydrolysis step”, complex substances originating in the feedstock are broken down to smaller fragments, whereby shorter carbohydrates, fatty acids and proteins result. The microorganisms that are involved in these reactions are strictly anaerobic. In the second step the resulted fragments are converted to shorter fatty acids like acetic acid, propionic acid and butyric acid respectively. Additionally lactic acid and alcohols are formed. This step is called the acidification step. The acidification step proceeds in parallel to the hydrolysis of the feedstock. During this step the strictly anaerobic microorganisms

gain energy for the first time. The third step is the so called acetogenesis step. Here the fatty acids and the other compounds are fermented to acetic acid and acetate respectively. The last step is the metanogenesis step. During this step the methane building microorgansims produce methane and carbon dioxide as well as hydrogen. When these four steps are operated in one fermenter, the process is called a single-stage process.

As mentioned above, the different microorganisms used for the methane production have different requirements for their habitat. Therefore, a compromise has to be made so that all of them can grow. Because the methane building microorganisms are the most sensitive bacteria in the process and only propagate slowly, the conditions of the processes are mostly adapted to them. As long as the oxygenation of the media is not too high, the bacteria of the first two process steps can consume the oxygen. Thus the methane bacteria, which need an oxygen free environment, are not harmed (KALTSCHMITT and HARTMANN, 2001).

2.7. The Becomix DH 1200 Homogenizer

In some experiments a homogenizer is used to reduce the size of the corn sillage.



model	output [l/min]		maximum pressure [bar]		drive	dimensions [mm]					
	pumping	dispersing	pumping	dispersing	[kW]	A	B	C	ØD	ØE	ØF
DH 1200	1600	900	3.0	2.2	22	885	470	590	125	80	25

Fig. 2.7.1 Becomix DH 1200 Duo-homogenizer

The Becomix DH 1200 Duo-homogenizer (fig. 2.7.1) similar to other inline homogenizers is equipped with a rotor-stator-system (fig. 2.7.2). The working principle of the rotor-stator-system can be described as follows:

The media (in this thesis a corn silage water slurry) is sucked into the rotor-stator-system. There it is deflected 90° and is transferred through the aperture of the

rotor. The rotor turns very fast with a circular velocity of 20m/s. The distance between the rotor and the stator is only a small gap. In this gap the material is accelerated. Because of the acceleration the solids of the slurry are impacted by very high shear stress. This high shear stress and collisions of the solids with the rotor-stator-system and among themselves cause a size reduction of the material. Additionally high turbulences appear which result in an optimal mixture of the slurry. Through apertures in the stator the homogenized slurry exits the Homogenizer.

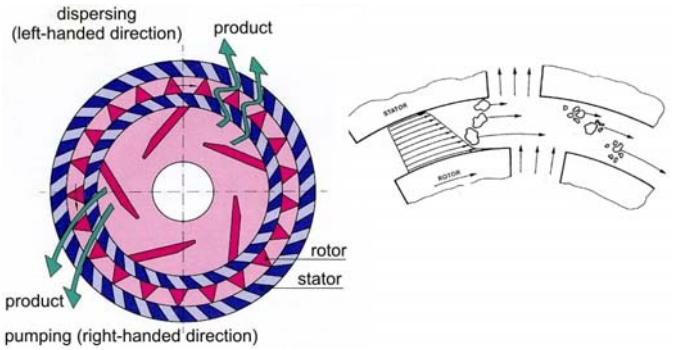


Fig. 2.7.2 Principle of the rotor-stator-system

2.8. The life cycle assessment

A life cycle assessment (LCA), which is also known as life cycle analysis, life cycle inventory, ecobalance or cradle-to-grave-analysis, is the assessment of the environmental impact of a given product throughout its lifespan. Products and services cause different energy and source demands and, therefore, produce different types of environment pollution during their preparation and Life cycle. These environment pollutions come from air, water and litter emissions respectively. The aim of LCA is to compare the environmental performance of the products and services and to be able to choose the least burdensome one, so that the stress to the sources and sinks can be minimised. To achieve this, useful models have to be developed and the material and energy alternation of the process has to be calculated and evaluated. Thus, the complete process has to be examined for air loading, water loading and soil loading. The assessment includes all process steps from the production of the raw material to the finished product. Additionally, the pollution caused by the usage of the product and the disposal of the disused product is also part of the analysis. Thus, all types of environment stress that are produced because of the product are examined and evaluated.

For LCA, first the initial situation has to be ascertained. In a second step a Life Cycle Inventory (LCI) has to be made. To create this LCI the boundaries of the system (scope) under consideration have to be set. Additionally a goal definition has to be made. The inventory of the initial situation is determined on the basis of these facts.

The goal definition of an LCA includes the description of the background, why the survey is made, what is determined and what the benefits are. By creating the scope of the system, all processes that belong to the system or do not belong to the system are defined. Additionally, the technical being applied has to be defined. Furthermore, the scope defines what kind of units [kg, m, J, etc.] are used to measure the data.

To describe the life cycle of the product, first a process flow chart containing all the processes during the life cycle of the product has to be made. This description of the system also contains the connection between all processes. The next step is to determine the values of all inputs and outputs of all the processes that occur during the product's life cycle and to create the LCI. Therefore, where possible, all material and energy flows are measured. Determining all the energy and material flows that enter or leave the system is only theoretical possible. The reason for this is that the enormous effort that is required to collect all the data for the survey exceeds financial budgets as well as the other available resources. Therefore, the boundaries set for the system which is to be assessed are limited. After all data for the LCA has been collected, the result of the balance is calculated and reported. For this thesis the tool GABI is being used.

3. Materials and methods

3.1. Raw materials

For the tests different raw materials containing starch (wheat, triticale or maize) or lignocellulosic biomass (corn silage) are used. In the following their characteristics and composition are described.

3.1.1. Wheat

Wheat (Triticum spp.) is a worldwide cultivated grass that comes from the region of the Near East. Beside maize and rice, wheat is the third most-produced cereal (FAO, 2007). Mostly wheat is used to produce flour for bakery products, to produce breakfast cereals and pasta. But wheat also is used to produce fermentation products like beer and vodka or to produce biofuels.

Wheat as complete genus has a wide diversity of species. Some wheat species are diploid, with two sets of chromosomes. But many wheat species are stable polyploids, with four sets of chromosomes (tetraploid) or have six sets of chromosomes (hexaploid). The origins of formal wheat cultivation lies in the nineteenth century, when single line varieties were created through selection of seed from a single plant noted to have desired properties. The major cultivation objectives that have been realised include high grain yield, good quality, disease and insect resistance and tolerance to abiotic stresses including mineral, moisture and heat tolerance.

Today the most common wheat is the species Triticum *aestivum* a hexaploid species. One thing that is quite special about wheat is that it is the only grain that contains gluten. By mixing wheat flour and water a visco-elastic dough can be made. This behaviour of the dough is based on the characteristic of the gluten combined with lipids contained in the wheat flour.

Water content [%]	Raw protein [%mas]	FS [%mas]
6.9-9,92	15,06	58,75

Table 3.1.1 Composition of wheat (raw protein based on DS; FS based on fresh matter)

3.1.2. Triticale

Triticale (x *Triticosecale*) is an artificial hybrid of rye (*Secale cereale* L.) as male and wheat (*Triticum aestivum* L.) as female hybrid partner. This grain was originally bred in Scotland and Sweden. Commercially available triticale is almost always a 2nd generation hybrid. Triticale combines the high yield potential and good grain quality of wheat with the high disease and environmental tolerance of rye. The word "Triticale" is a fusion of the latin words *triticum* (wheat) and *secale* (rye). When Triticale is cultivated under optimum conditions it is possible to obtain nearly the same yields per hectare as for wheat cultivation. The type of triticale which was used for the experiments is Lamberto. The triticale was cultivated at the Ihinger Hof of the University of Hohenheim (Stuttgart, Germany) and harvested in 2004. The manuring was divided into 2 ferilisations. The first manuring (60%) was performed at the time of the sowing and the second manuring (40%) was performed during the raking (further details: http://www.uni-hohenheim.de/Pflanzenbau/lehre/versuchsfeld2005/versuchsfeldfuehrer2005band1.pdf).

Water content [%]	FS [%mas]
10.24	65.77

Table 3.1.2 Composition of triticale (FS based on fresh matter)

3.1.3. Maize

The term maize derives from the Spanish form of the indigenous Taino word for the plant, maíz. Maize (*Zea mays L*. ssp. mays) which in most english speaking countries also is known as corn, is a grass domesticated in Mesoamerica. There are some reports that the Spanish first grew maize in southern Mexico. Today maize is spread throughout the American continents as the largest crop there. After European contact with the Americas in the late 15th and early 16th centuries, maize spread to the rest of the world.

Most of the farmers grow hybrid maize, because of its high grain yield as a result of heterosis compared to conventional varieties. Maize only can be produced in areas that do not have extreme cold temperatures, as it is a cold-intolerant crop. It is a facultative long-night plant and flowers in a certain number of growing degree

days with temperatures higher than 10 °C. As a C4 plant (a plant that uses C4 carbon fixation), maize is a considerably more water-efficient crop than C3 plants (plants that use C3 carbon fixation) like the small grains, alfalfa and soybeans. Maize is most sensitive to drought at the time of silk emergence, when the flowers are ready for pollination. Maize has grains that have about the size of peas that adhere in regular rows round a white pithy substance the panicle. The complete arrangement of the grains round the panicle forms the ear. An ear contains approx. 200 to 400 kernels and has a length of about 10–25 cm. When maize grain is ground into flour it yields more flour with less bran than wheat. In difference to wheat corn grains lack the protein gluten. Therefore Corn makes baked goods with poor rising capability and coherence.

Water content [%]	Raw protein [%mas]	FS [%mas]
9.92	10.51	64.87

Table 3.1.3 Composition of maize (raw protein based on DS; FS based on fresh matter)

3.1.4. Corn Silage

Normally silage is a fermented, high-moisture forage which is fed to ruminants, cud-chewing animals such as cattle and sheep. Silage is mostly made from grass crops such as maize. It is made from the entire plant and not only the grain. Corn silage must be made from plant material with an adequate moisture content, which ranges from approximately 55% to 70%, depending on the construction of the storage structure and, hence, the degree of compression and the amount of water that will be lost during storage. In the case of corn silage the harvest of the entire maize plant begins when the moisture is at a suitable level. The plant material is collected, chopped into pieces ca. 13 mm long and packed into the storage. Afterwards the chopped material is compressed in a clamp silo (fig. 3.1.1) by a tractor so that it is hermetically sealed stored in an air-tight manner.

This air-tightness ensures that the plant's enzymes as well as aerobic and facultative anaerobic bacteria, yeasts and moulds are suppressed. Lactic acid bacteria metabolise the sugars present in the sap into acid and the pH decreases to ca. 4.0 to 4.5. At this pH other harmful fermentation disturbing bacteria such as *Coli*, *Listeria* and *Clostridia* species are inhibited.

Fig. 3.1.1 Clamp silo (source: http://commons.wikimedia.org/wiki/Image:Fahrsilo.jpg)

The corn silage used for the experiments is produced of the maize type Benicia that was cultivated at the Universitiy of Hohenheim (Stuttgart, Germany). The silage has the corn silage maturation group S 280. This is the category for medium late silage. The average distance between the rows of maize was ca. 75cm. Approximately 10 grains per square metre were sown. The grain was sown in field M8 on 22.04.2003. The maize was harvested on 25.08.2003 (approx. 3-4 weeks earlier than usual, because of the dry summer in 2003). The maize was manured with approx. 200kg/ha N overall. The manuring was divided into 2 fertilisations. The first manuring took place at the time of sowing and the second manuring was performed at the raking.

Based on dry substance, the different corn silage samples used for the experiments had a composition of:

Cellulose [%]	Xylan [%]	Arabinan [%]	Starch [%]	acid soluble lignin
25-31	13-17	3.1-4.1	18.6-23.1	7.2

Table 3.1.4 Composition of different corn silage samples (all data is based on DS)

The dry substance content (DS) of the different samples varied between 25 and 30%.

3.2. Enzymes

3.2.1. Overview of all enzymes used for the experiments

For all experiments different enzymes or enzyme systems are used. In table 3.2.1 their name, source, manufacturer and action are described.

Enzyme	Source	Manufacturer	Enzyme action
Gammaclast 2OP	*Aspergillus spec.*	Gamma Chemie, Darmstadt	Alpha-amylase
GC 106	*Aspergillus niger*	Genencor, Leiden	Low pH protease (AHMED and TRAISTARU, 2004)
GC 220	*Trichoderma reesei*	Genencor, Leiden	cellulase enzyme complex containing cellobiase and cellobiohydrolase I activity (KABEL et al., 2006)
GC 626	*Aspergillus kawachi*	Genencor, Leiden	granular starch hydrolyzing Alpha-amylase
GC 880	*Trichoderma reesei*	Genencor, Leiden	cellulase enzyme complex containing β-D-glucanase and β-D-xylanase (SCHOBER, 2008)
Liquozyme 280L	*Bacillus licheniformis*	Novozymes, Bagsvaerd	Thermostable bacteria amylase
Optimash BG	*Trichoderma reesei*	Genencor, Leiden	hemicellulase enzyme complex containing ß-glucanase and xylanase activity
SAN Super	*Aspergillus* niger	Novozymes, Bagsvaerd	Amyolytic enzyme complex containing Alpha-amylase, amyloglucosidase and protease enzyme activity
STARGEN 001	*Aspergillus* kawachi, *A.niger*	Genencor, Leiden	α-amylase from *Aspergillus* kawachi and glucoamylase from A. niger.

Table 3.2.1 Enzymes used during the experiments

3.3. Analytical Methods

3.3.1. Determination of structural carbohydrates in biomass

The Method used is based on the laboratory analytical procedure “Determination of Structural Carbohydrates and Lignin in Biomass” of the NREL (National Renewable Energy Laboratory; Department of Energy, United States of America). To perform this analysis following apparatus are needed:

- Analytical balance, accurate to 0.1mg
- Drying oven with temperature control of 105°C ± 3°C
- Glass pans for analysis of dry substance (DS)
- Water bath, set at 30°C ± 3°C
- Autoclave, set to 121°C ± 3°C
- Desiccator containing desiccant
- HPLC system equipped with:
    - Refractive index detector (ERC 7510, Bischoff, Leonberg Germany)
    - RHM monosaccharide column (Phenomenex, Aschaffenburg, Germany)
    - Autosampler (728, Bischoff, Leonberg, Germany)
    - Column thermostat (Jetstream 2 PLUS, Bischoff, Leonberg, Germany)
- Test tubes with threat (98 x 16mm) and plastic screw-cap (3.561 103 , Schütt Labortechnik GmbH, Göttingen, Germany)
- Vortexmixer
- Syringes fitted with 0.45µm syringe filters (7-8703, 25mm RC filter, Neo Lab, Heidelberg, Germany)

Additionally, following reagents are needed:

- Sulfuric acid, 72% w/w (Merck KGaA, Darmstadt, Germany)
- Calcium carbonate (Carl Roth GmbH, Karlsruhe, Germany)
- Purified water
- Standards of:
    - Cellobiose (Carl Roth GmbH, Karsruhe, Germany)
    - D(+)glucose (Serva Electrophoresis GmbH, Heidelberg, Germany)
    - D(+)xylose (Merck KGaA, Darmstadt, Germany)
    - D(+)galaktose (Serva Electrophoresis GmbH, Heidelberg, Germany)
    - D(-)arabinose (Sigmachemie, Steinheim, Germany)
    - D(+)mannose (Carl Roth GmbH, Karsruhe, Germany)

To perform the analysis firstly 300 mg of the biomass is weighed out as sample into a test tube. The amount weighed out has to be recorded to the nearest of

0,1mg. Each sample should at least be run in duplicate. Then 3.00 ml (or 4.92 g) 72% sulphuric acid have to be added to each sample, which then has to be sealed with a plastic screwcap. To make sure that all samples are mixed well, each sample is homogenized using the vortex mixer thoroughly. To moisten the material, all the test tubes have to be placed in a water bath which was set at 30°C. There the samples were incubated for 60 min. Every 5 min the samples were stirred with the vortex mixer to keep them homogenous. Stirring the samples is essential to ensure that every particle is contacted by the acid and that a uniform hydrolysis is accomplished. After the 60-minute hydrolysis the test tubes have to be removed from the water bath to stop the moistening. Then the acid concentration has to be diluted to a 4% concentration by adding 84ml (or 84g) deionised water to every test tube. To ensure that the concentration of the complete sample is the same, all samples have to be mixed. In addition to the normal samples, a set of sugar recovery standards (SRS) that will be treated like the biomass samples are prepared as well. The SRS will be used to compensate losses due to destruction of sugars during the hydrolysis of the samples. SRS should include D(+)glucose, D(+)xylose, D(+)galaktose, D(-)arabinose and D(+)mannose. To prepare the SRS proceed as for the process described above for biomass samples. Then place all test tubes into an autoclave and autoclave the sealed samples and SRS for one hour at 121°C. After the completion of the one hour, allow the samples to cool down to room temperature before removing the screwcaps from the test tubes. Parallel to the analysis of the structural carbohydrates, an analysis of the dry substance (DS) of the biomass sample has to be made. Therefore, the sample has to be weighed in a pre-dried glass pan (dried for at least 4 hours at 105°C). Record the weight of the glass pan and the biomass sample which was used for the DS analysis. Then place the sample in a convection oven set to 105°C and dry to a constant weight (at least 4 hours). Record the constant weight of the glass pan and the dried biomass sample.

To determine the content of structural carbohydrates, firstly every sample and the SRS respectively have to be neutralized to pH 5-6 by adding calcium carbonate. Then, to prepare the samples for HPLC analysis, every neutralized sample has to be filtered through a 0.45µm syringe filter into an auto sampler vial. Then they are analysed using a HPLC system.

- HPLC conditions:
  - Flow rate 0.6ml/min
  - Mobile phase 0.01n H_2SO_4
  - Column temperature 80°C
  - Detector temperature 35°C
  - Run time 35 min

Calculations:

$$\%\,\text{sugar} = \frac{\left(\frac{C_{HPLC}\left[\frac{mg}{ml}\right]}{\frac{\%R_{sugar}}{100}} \cdot \text{Anhydro correction} \right) \cdot 87\text{ml} \cdot \frac{1\text{g}}{1000\text{mg}}}{\text{weight}_{sample}\,[\text{g}] \cdot \frac{DS\%}{100\%}} \cdot 100$$

Formula 3.3.1 Calculation of the percental sugar content

where:

$$\%R_{sugarr} = \frac{c_{sugar,\,HPLC}\left[\frac{mg}{ml}\right]}{c_{sugar,\,initial\ weight}\left[\frac{mg}{ml}\right]} \cdot 100$$

Formula 3.3.2 Percental average recovery of sugar recovery standards

  - where:
    - $c_{sugar,\,HPLC}$ = concentration of SRS standard measured in HPLC
    - $c_{sugar,\,initial\ weight}$ = weight of SRS standard used for sugar recovery analysis in [mg/ml]
- c_{HPLC} = concentration determined in HPLC [mg/ml]
- $\%R_{sugar}$ = average recovery of sugar recovery standards (SRS) [%]
- Anhydro correction: to calculate the polymeric sugars from the concentration of the corresponding monomeric sugars, 0.88 (or 132/150) is used for C_5-sugars (Xylose, Arabinose) and 0.9 (or 162/180) is used for C_6-sugars (Glucose, Galactose, Mannose)
- weight $_{sample}$ = weight biomass sample for analysis [g]

– $$DS\% = \frac{\text{weight}_{\text{dry pan plus sample}} - \text{weight}_{\text{dry pan}}}{\text{weight}_{\text{sample as received}}} \cdot 100$$

Formula 3.3.3 Percental dry substance content

- where:
  - $\text{weight}_{\text{dry pan plus sample}}$ = weight of glass pan for DS analysis and analysed sample when constant weight is obtained
  - $\text{weight}_{\text{dry pan}}$ = weight of glass pan for DS analysis
  - $\text{weight}_{\text{sample as received}}$ = weight of biomass weighed into glass pan

3.3.2. Determination of acid soluble lignin in biomass

To analyse the acid soluble lignin in biomass, the hydrolysis liquor which was obtained performing the structural carbohydrates analysis method can be used. The amount of acid soluble lignin can then be analysed easily using a UV-Vis spectrometer. The 4% sulfuric acid sample has to be measured against the blank of a pure 4% sulfuric acid. The sample has to be diluted so that the absorbance is in the range of 0-0.8. If the sample is diluted, the same dilution of the acid has to be done to measure the blank. Each sample should be measured at least in duplicate.

Apparatus:

- UV-Vis spectrometer (Beckman Coulter DU 640, Krefeld, Germany)
- UV Cells (104 QX 10mm Suprasil 300, Hellma GmbH & Co KG, Müllheim, Germany)

Calculation of acid soluble lignin:

$$\%\,\text{ASL} = \frac{\text{UV}_{\text{abs}} \cdot 87\text{ml} \cdot \text{Dilution}}{\text{weight}_{\text{sample}}\,[\text{g}] \cdot \frac{DS\%}{100\%} \cdot \varepsilon} \cdot 100$$

Formula 3.3.4 Percental content of acid soluble lignian

where:

- %ASL = acid soluble lignin [%]
- UV_{abs} = absorbance measured
- Dilution = dilution so that the absorbance is in the range between 0 and 0.8.
- weight $_{sample}$ = weight biomass sample for analysis [g]
- ε = absorptivity constant for acid soluble lignin
    - wave length 320nm: ε = 30 L/g · cm (cp. data NREL: for corn stover) was used
- $$DS\% = \frac{\text{weight}_{\text{dry pan plus sample}} - \text{weight}_{\text{dry pan}}}{\text{weight}_{\text{sample as received}}} \cdot 100$$

Formula 3.3.5 Percental dry substance content

- where:
    - $weight_{dry\ pan\ plus\ sample}$ = weight of glass pan for DS analysis and analysed sample when constant weight is received
    - $weight_{dry\ pan}$ = weight of glass pan for DS analysis
    - $weight_{sample\ as\ received}$ = weight of biomass weighed into glass pan

3.3.3. Determination of Furfural and Hydroxymethylfurfural (HMF) as degradation products in pre-treated biomass slurries

The method used is based on the laboratory analytical procedure “Determination of Sugars, and Degradation Products in Liquid Fraction Process Samples” of the NREL (National Renewable Energy Laboratory; Department of Energy, United States of America).

To perform this analysis, following apparatus are needed:

- HPLC system equipped with:
    - Refractive index detector (ERC 7510, Bischoff, Leonberg Germany)

  - RHM monosaccharide column (Phenomenex, Aschaffenburg, Germany)
  - Autosampler (728, Bischoff, Leonberg, Germany)
  - Column thermostat (Jetstream 2 PLUS, Bischoff, Leonberg, Germany)
- Syringes fitted with 0.45µm syringe filters (7-8703, 25mm RC filter, Neo Lab, Heidelberg, Germany)

Additionally following reagents are needed:

- Standards of:
  - 5-hydroxy-2-furaldhyde (HMF)
  - Furfural
  - Lactic acid
  - Acetic Acid
  - Ethanol

Firstly, to perform the analysis an aliquot of a pre-treated biomass sample is taken and diluted 1:9 (resulting dilution factor is 10) with distilled water. Then the diluted sample is filtered through a 0.45µm syringe filter into an auto sampler vial and analysed using a HPLC system.

Component	Approximate retention time [min]	Concentration range of standards [g/l]
Lactic Acid	13.6	0.11-10.7
Acetic Acid	15.8	0.12-12.5
Ethanol	22.5	0.1-9.4
HMF	30.8	0.02-1.8
Furfural	46.7	0.05-4.6

Table 3.3.1 Retention time and concentrations for different HPLC standards

- HPLC conditions:
  - Flow rate 0.6ml/min
  - Mobile phase 0.01n H_2SO_4
  - Column temperature 55°C
  - Detector temperature 35°C

- Run time 60 min

3.3.4. The determination of the dry substance content (DS)

Dry substance (DS) is the sum of all non volatile components (MATISSEK et al, 1992). The DS is examined by drying of the sample to a constant weight at 105°C and weighing the humid and the dry sample. To determine the DS of a sample ca. 2-5g of homogenized sample are weighed in into a glass crucible. Then the sample is dried over night in a drying cabinet at 105°C. Afterwards the sample is cooled down in a desiccator to room temperature before the weight of the residue can be measured.
Calculation of the proportional DS content:

$$DS = \frac{m_3 - m_1}{m_2 - m_1} \cdot 100$$

Formula 3.3.6 Percental dry substance content

DS = dry substance
m_1 = weight of empty glass crucible
m_2 = weight of empty glass crucible and sample before drying
m_3 = weight of empty glass crucible and sample after drying

3.3.5. The determination of the free amino nitrogen (FAN)

Low-molecular amino molecules like amino acids have a great influence on the progress and the by-products that are generated during fermentation. The concentration and the mixture of the amino acids have an influence on the redox potential because of components that can result from chemical reactions during the mashing.
Besides quantitative methods of single amino acids (Ion exchange methods, HPLC methods, GC methods), summary methods, which also detect ammonia ions (NH_4^+) and some amines, are used.
When using methods that are based on colour reactions, different amino acids appear with different colour intensities. Usually for such methods a standard amino acid, mostly Glycin, is used. For this method Ninhydrin is used as reactant for the

colour reaction. Based on Glycin as standard the colour intensity of different amino acids varies between 70 to 105%. The method detects amino acids, ammonia and end terminal α-amino groups of peptides and proteins.

Apparatus

- Test tubes with threat (98 x 16mm) and plastic screw-cap (3.561 103 , Schütt Labortechnik GmbH, Göttingen, Germany)
- Vortexmixer
- Water bath with boiling water
- Water bath at 20°C
- UV-Vis spectrometer (Beckman Coulter DU 640, Krefeld, Germany)
- 10mm Cuvettes

Chemical reaction

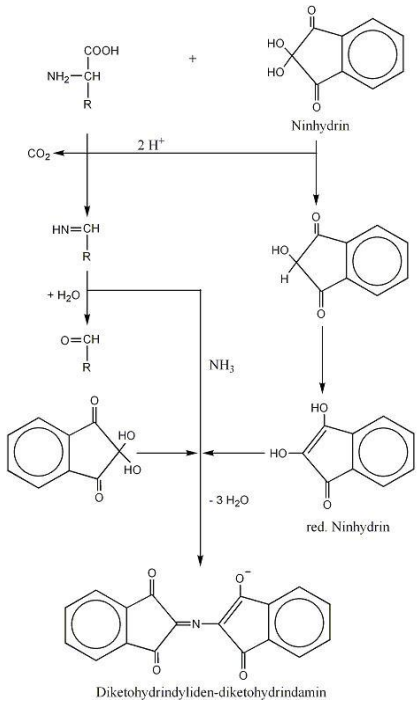


Fig. 3.3.1 Chemical reaction of Ninhydrin with amino acids

Reagents

Colour reagent:

- 10.0g Di-Sodium-hydrogenphosphate (Na_2HPO_4 * 12 H_2O)

- 6.0g potassium-dihydrogen phosphate (KH_2PO_4)
- 0.5g Ninhydrin
- 0.3g Fructose

All chemicals have to be mixed throughly with water. Afterwards the mixture has to be topped up to 100ml (dark bottle, pH 6.6-6.8).

Diluting solution:

- 2g Potassioniodat dissolved in 600ml of water and 400ml of ethanol (96%)

Stock solution:

- 107.2mg of Glycin dissolved in 100ml of water (storage at 0°C)

Standard solution:

- 1ml of stock solution mixed with water and filled up to 100ml (the standard solution contains 2ml/l of amino nitrogen)

Procedure

- Dilute the sample to a level of about 1 – 3mg/l of amino nitrogen
- Sample, standard solution and blank value (water) have to be messured at least in triplicate
- 2ml of the diluted sample, standard solution and blank value respectively have to be transferred into a test tube
- Add 1ml of the colour reagent and mix properly
- Lock test tube to reduce evaporation loss
- Heat for 16min in boiling water bath
- Cool for 20min in water bath at 20°C
- Add 5ml of the Diluting solution
- Measure extinction within 30min in a 10mm cuvette in comparison to water

Calculation

$$FAN = \frac{E_P - E_B}{E_S} \cdot 2 \cdot F$$

Formula 3.3.7 Calculation of the FAN in [mg/l] without decimal

- where:
  - E_P = average extinction of the samples
  - E_S = average extinction of the standard solution
  - E_B = average extinction of blank
  - F = dilution of the sample
  - 2 = concentration of the Glycin standard solution [mg/l]

3.4. Hydrolysis of lignocellulosic biomass (e.g. corn silage)

As already described before, the digestion of lignocellulosic biomass, which is a relatively rigid material, is much more complex than the normal starch to ethanol process that usually is used in distilleries. To hydrolyse this very rigid material first of all a pre-treatment, helping to make the material more accessible to enzymes, has to be done. To find the conditions that are optimal for a process using the equipment of regional plants, pre-treatment tests at feasible temperatures have to be performed.

3.4.1. The influence of the pre-treatment conditions using direct heating for pre-treatment on the enzymatic digestibility of corn silage

As already mentioned in chapter 2, the biomass has to be pre-treated to make the cellulose accessible for the enzymatic hydrolysis. In the experiments to be described here, the raw material (corn silage) is pre-treated in a temperature controlled jet cooker which is heated by a jet pump. Before the corn silage is heated, 10kg corn silage and 80kg tap water are pumped together into the jet cooker. Then the mixture has to be stirred using a propeller stirrer and, simultaneously, heated up to the required pre-treatment temperature. When the temperature is reached, the pre-treatment started. In this series of experiments temperatures between 135 and 160°C are used to make the corn silage more accessible. At these temperatures dwell periods of 15 to 90min are used to determine the optimum temperature and dwell period for the pre-treatment of the raw material. After the heating period has been over, the slurry is steam exploded,

which means transferred by spontaneous pressure drop, into a conventional distillery mash vessel that is larger in volume than the slurry needed. Ca. 20 min later, when the slurry has cooled down a bit, everything is pumped completely into a plastic vessel which contains a sieve at the lower head to restrain the fibres. The liquid phase of the slurry, which passes the sieve, is collected in a plastic vessel installed below. Fig. 3.4.1 shows the arrangement for the pre-treatment experiments using direct heating with a jet pump.

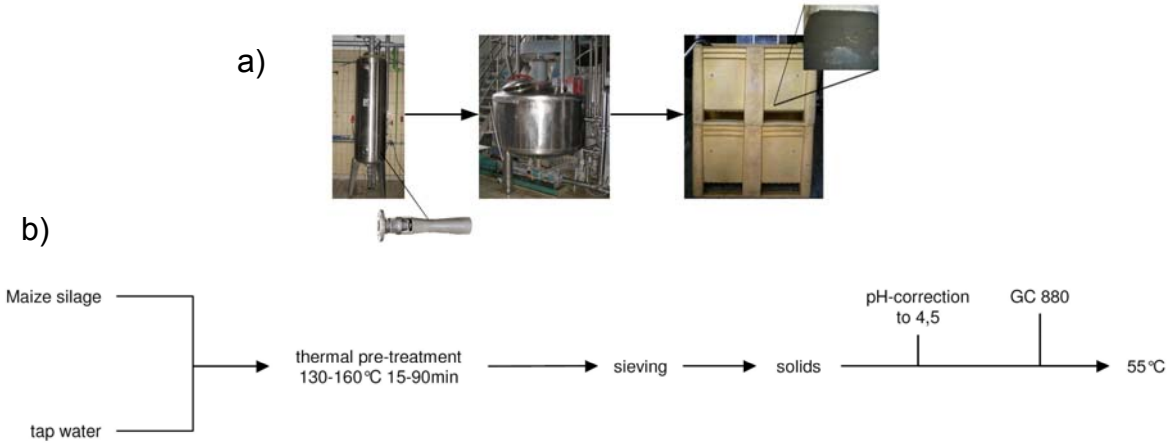


Fig. 3.4.1 a) Flow chart of the pre-treatment of corn silage using direct heating. b) Flow chart of the experiments using direct heating for pre-treatment of corn silage before the enzymatical hydrolysis

The resulted fibres are frozen at -18°C to store them until they are needed. To test which pre-treatment process worked the best, it is followed by an enzymatic hydrolysis process at the pH of 4.5 and 55°C. The mashes that are prepared all contained 9.5% of DS except those where it is mentioned. The dosage of the enzyme GC 880 is set to 165.79µl/g DS (ca. 1.1kU/g DS and 8.5mg protein/g DS respectively). When the temperature of 55°C is reached, the enzyme is added. At this moment the hydrolysis period of ca. 48h started. During the hydrolysis different samples are taken and measured using the HPLC. To prepare the samples for HPLC analysis the samples are filtered through a 0.45µm syringe filter into an autosampler vial.

HPLC conditions:

- Flow rate 0.6ml/min
- Mobile phase 0.01n H2SO4

- Column temperature 50°C
- Detector temperature 35°C
- Run time 35 min

The samples are analysed in the same HPLC system that is already mentioned before in chapter 3.2.1.

- Refractive index detector (ERC 7510, Bischoff, Leonberg Germany)
- RHM Monosaccharide column (Phenomenex, Aschaffenburg, Germany)
- Auto sampler (728, Bischoff, Leonberg, Germany)
- Column thermostat (Jetstream 2 PLUS, Bischoff, Leonberg, Germany)

To decide how effective the pre-treatment method is, the glucose concentration is calculated as proportional glucan yield after 48h of hydrolysis.

3.4.2. The influence of dispersing the corn silage before pre-treatment on its enzymatic digestibility

In analogy to the experiments that used direct steam addition for the pre-treatment step, these experiments are also carried out using direct heating for the pre-treatment. Unlike to the tests that had been made before, these tests used dispersed corn silage as the raw material. To reduce the size of the corn silage (quadratic pieces of ca. 1cm in length per side) a Becomix DH 1220 homogenizer (Berents GmbH, 28816 Stuhr, Germany) is used. Slurries containing ca. 6 to 7 % DS are prepared so that the viscosity would not get too high during the dispersing process and the homogenizer would not get blocked. In fig. 3.4.2 a picture of the arrangement of the equipment for homogenizing the biomass can be seen. The corn silage has to be mixed together with tap water in a high-grade steel vessel before the homogenizer sucked the mixture into its dispersing tool. During the tests a rotation speed of 15m/s is used. The homogenized slurry is then transferred into a conventional stillage sieve to separate the solid fibres from the process water. The separated process water is recycled into the high-grade steel vessel to process further corn silage. This has to be done to save water throughout the total distillery process. The dispersed corn silage that could be produced using such a process results in ca. 15% DS and smaller fibres. To

examine the effect that could be achieved using the homogenizer experiments using the ca. 125kg slurry resulted plus 5kg tap water that is needed to rinse the feeding pipe of the jet cooker has to be pumped into the jet cooker.

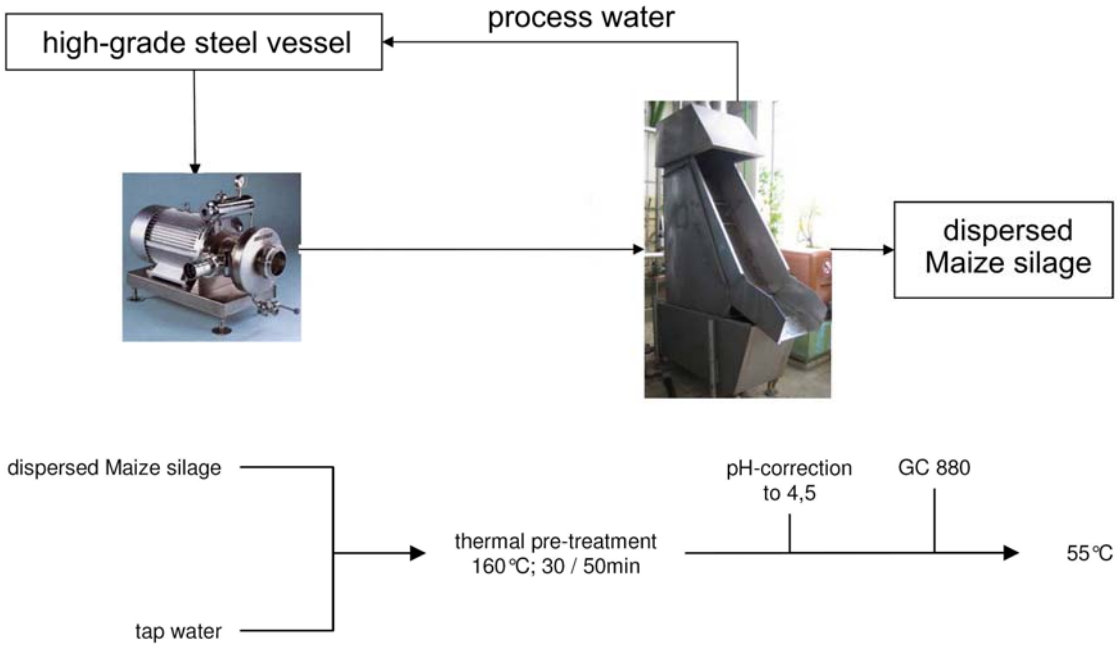


Fig. 3.4.2 Flow chart for the dispersing of corn silage using a Becomix DH 1200 (Berents GmbH, 28816 Stuhr, Germany)

Then pre-treatment experiments similar to those described in chapter 3.3.1 (The influence of the pre-treatment conditions on the enzymatic digestibility of corn silage using direct heating for pre-treatment) are performed. But instead of sieving the resulting slurry, it is used as received from the process. After the pre-treatment using direct heating with steam, laboratory tests are made. The mashes that are prepared contained between 5.27and 6.5% DS respectively. Because of the best results previously achieved using 160°C as pre-treatment temperature, the tests are performed at this temperature. Because of the smaller size of the fibre particles, shorter dwell periods for the pre-treatment step are chosen to try to minimise sugar losses. The dosage of the enzyme GC 880 is set to 180.65µl/g DS (ca. 1.1kU/g DS and 8.26mg protein/g DS respectively). When the temperature of 55°C has been reached, the enzyme is added. At this moment the hydrolysis period starts. During the hydrolysis various samples are taken and measured using the HPLC. To prepare the samples for HPLC analysis, the samples are filtered through a 0.45µm syringe filter into an autosampler vial.

HPLC conditions:

- Flow rate 0.6ml/min
- Mobile phase 0.01n H2SO4
- Column temperature 50°C
- Detector temperature 35°C
- Run time 35 min

The samples are analysed in the same HPLC system that is already mentioned before.

- Refractive index detector (ERC 7510, Bischoff, Leonberg Germany)
- RHM monosaccharide column (Phenomenex, Aschaffenburg, Germany)
- Auto sampler (728, Bischoff, Leonberg, Germany)
- Column thermostat (Jetstream 2 PLUS, Bischoff, Leonberg, Germany)

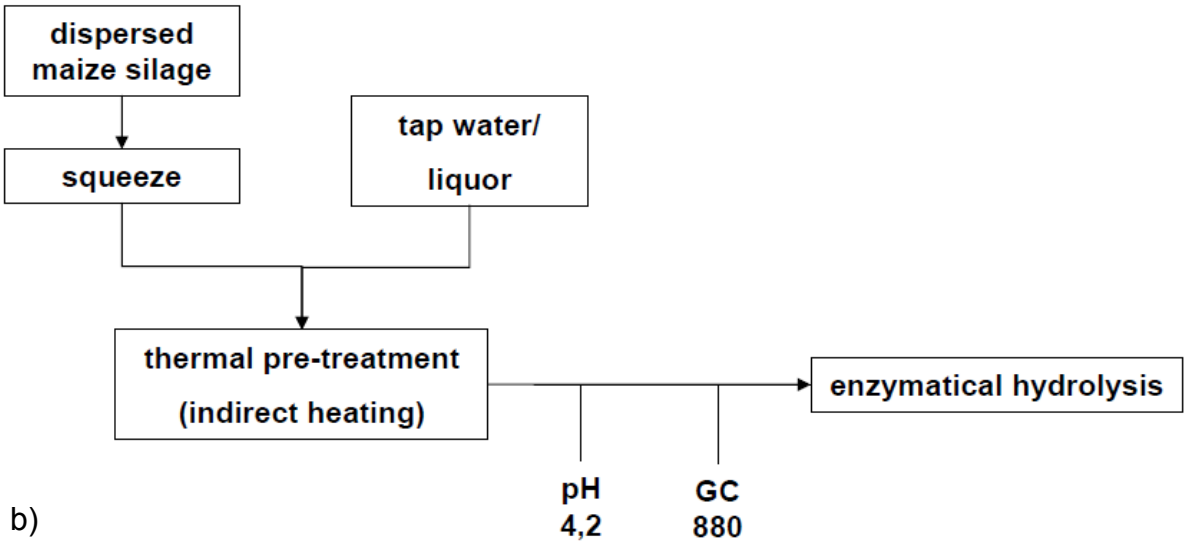
To decide how effective the pre-treatment method has been, the resulting glucose concentration is calculated as proportional glucan yield after 48h hydrolysis.

3.4.3. The influence of the pre-treatment conditions on the enzymatic hydrolysis of dispersed corn silage using indirect heating for pre-treatment

As already described in chapter 3.4.2 (The influence of dispersing the corn silage before pre-treatment on its enzymatic digestibility), the corn silage is dispersed before the pre-treatment. But, unlike to these experiments, the dispersed raw material is pressed first using a squeezer to remove more water from the fibres. After removing the water, a DS of ca. 40% is obtained. To avoid rotting of the material, the fibres resulted are frozen at -18°C to store them until they are needed for pre-treatment tests. Before the corn silage is heated 2.5kg of pressed corn silage are weighed in and 4.5kg tap water or water removed from the squeezer are added so that the DS of the mixture should be ca. 14-15%DS as before squeezing of the material. For other experiments at higher DS contents 3kg of the dispersed material are mixed together with 3kg of water. Then the slurry is transferred into a jet cooker which could be heated indirectly using a heating jacket (fig. 3.4.3). The

advantage of the indirect heating during the pre-treatment process is that no steam could condense to water in the slurry and, therefore, the DS could not decrease as in the experiments using direct heating. When the mixture has been transferred into the jet cooker all outlets are closed, and then the stirrer and the steam pre-treatment are started. After ca. 5min of heating, the chosen pre-treatment temperature is reached. At this moment the dwell period of the pre-treatment process starts. When the pre-treatment process is finished, the complete slurry is steam exploded into a high-grade vessel that was placed under the jet cooker. To ensure that the slurries did not rot, the pre-treated raw material obtained, is stored at -18°C until it is needed for enzymatic laboratory digestion experiments.

a)



b)

Fig. 3.4.3 a) Jet cooker using heating jacket for indirect heating of the biomass. b) Flow chart of the experiments using indirect heating for pre-treatment before enzymatical hydrolysis of corn silage

Before these experiments are made, the DS of the biomass is determined so that the right dosage of enzyme (GC 880) per gramme of dry substance could be added. As with the experiments using direct heating, ca. 180.47µl GC880/g DS, that corresponds to 8.65mg protein/g DS or ca. 1.15kU/g DS, are used to get comparable results. The defrosted corn silage is weighed into stainless steel cans. Because the resulting pH from the pre-treatment is ca. 4.2, the experiments are performed without any correction of the pH. Then the temperature is adjusted to 55°C. After reaching 55°C, the cellulase complex GC 880 (Genencor International, Leiden, Netherlands) is added to start the enzymatic hydrolysis. During the stirring of the mashes at 100 rpm in the stainless steel vessels with a Heidolph laboratory stirrer (RZR 2020, Heidolph Instruments GmbH & Co KG, D-91126 Schwabach, Germany) and a propeller stirrer (Roth 0867.1, 50mm, Roth D-76231 Karlsruhe, Germany) samples are taken at different times to be analysed using HPLC to get an idea of the progress of the glucan hydrolysis. To prepare the samples for HPLC analysis, the sample has to be filtered using a plastic syringe and a 0.45µm sterile filter.

The HPLC analysis is performed using a Bischoff HPLC system which is equipped with a:

- Refractive index detector (ERC 7510, Bischoff, Leonberg Germany)
- RHM monosaccharide column (Phenomenex, Aschaffenburg, Germany)
- Auto sampler (728, Bischoff, Leonberg, Germany)
- Column thermostat (Jetstream 2 PLUS, Bischoff, Leonberg, Germany)

- HPLC conditions:
  - Flow rate 0.6ml/min
  - Mobile phase 0.01n H_2SO_4
  - Column temperature 80°C
  - Detector temperature 35°C
  - Run time 30 min

3.4.4. Optimization of the enzyme dosage of the cellulase complex GC 220 for enzymatic hydrolysis

Because the samples of the cellulase complex GC 880 used before ran out, a commercially available cellulase complex called GC 220 (Genencor, Leiden, Netherlands) has been tested for its effectiveness on pre-treated biomass. In the following tests, the influence of different GC 220 dosages, with a minimum enzyme

activity of 6200 IU/g (data sheet from Genencor), on biomass is tested. To perform these tests, dispersed corn silage is heated indirectly at 150°C for 60min. After pre-treatment the material has a DS of 12.25%. To test the influence of the dosage of GC 220, enzyme loadings of 180µl, 200µl, 250µl and 300µl/g DS are used. The mashes are prepared by weighing 500g of the pre-treated biomass into stainless steel cans (Edelstahl Dose mit Deckel, 2500ml, 1-1185, NeoLab, D-69123 Heidelberg, Germany). The pH of the material is ca. 4.2 and, analogous to the experiments using GC 880, the pH is not changed at all. Then the mashes are heated up to 55°C and stirred at 100 rpm in the stainless steel cans using a Heidolph laboratory stirrer (RZR 2020, Heidolph Instruments GmbH & Co KG, D-91126 Schwabach, Germany) and a propeller stirrer (Roth 0867.1, 50mm, Roth D-76231 Karlsruhe, Germany). When the temperature of 55°C has been reached, the different dosages as mentioned above are added. At this time the enzymatic hydrolysis starts. At various intervals samples are taken and prepared for HPLC analysis filtering them using a plastic syringe and a 0.45µm sterile filter.
The HPLC analysis is performed using a Bischoff HPLC system which is equipped with a:

- Refractive index detector (ERC 7510, Bischoff, Leonberg Germany)
- RHM monosaccharide column (Phenomenex, Aschaffenburg, Germany)
- Auto sampler (728, Bischoff, Leonberg, Germany)
- Column thermostat (Jetstream 2 PLUS, Bischoff, Leonberg, Germany)

- HPLC conditions:
  - Flow rate 0.6ml/min
  - Mobile phase 0.01n H_2SO_4
  - Column temperature 80°C
  - Detector temperature 35°C
  - Run time 30 min

3.4.5. Experiments using lignocellulosic biomass (corn silage) and starchy material (triticale) in one process to produce ethanol

Because of the problem that distillery processes using only lignocellulosic biomass to produce ethanol normally result in ethanol concentrations <6%vol., a process combining the utilization of starchy and lignocellulosic raw material is to be tested. To obtain such a distillery process using cellulosic biomass as well as starchy material for ethanol production, one possibility to produce ethanol of both is to

combine the mash process for both materials. Following experiments are performed to assess the impact of different pre-treatment parameters (temperature and dwell period) and enzyme dosages.

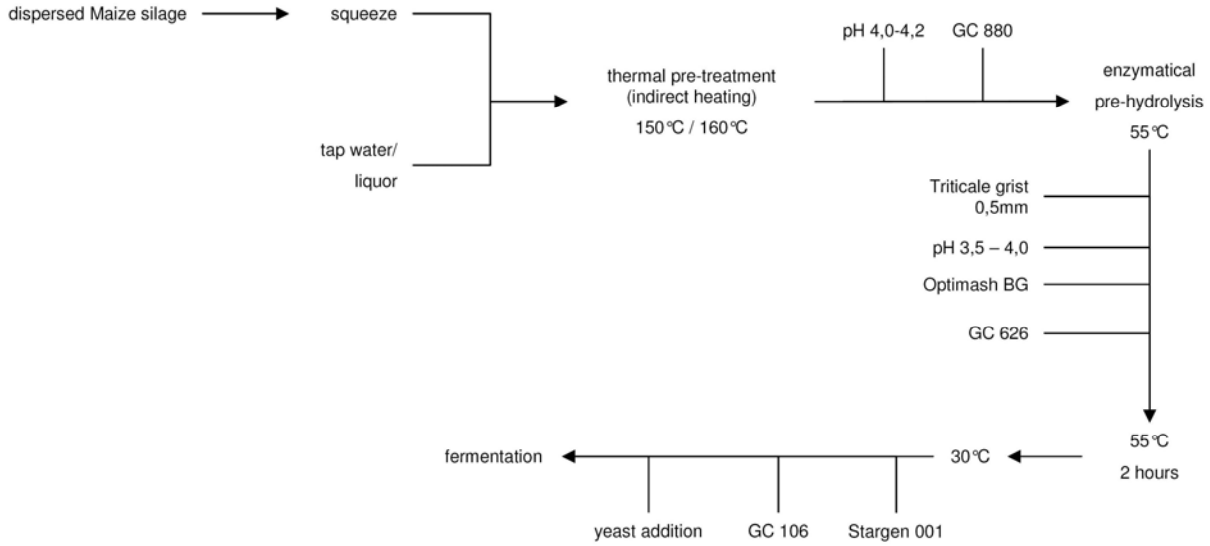


Fig. 3.4.4 Flow chart of the combined SSF experiments using indirect heating for the pre-treatment of dispersed corn silage

In a first stage the cellulosic material has to be pre-treated as described in chapter 3.4.3. To prevent a high decrease in dry substance for the further process the pre-treatment stage is performed using indirect heating. Considering the best results of the tests of the direct heated and dispersed corn silage the temperatures of 150 and 160°C are used in those experiments to pre-treat the material. The dwell period for the experiments at 160°C is 30minutes. Additional experiments at 150°C using a dwell period of 30, 40 and 50 minutes respectively are performed. After the dwell period is over the pre-treated corn silage is steam exploded into a stainless steel vessel. To know how much enzyme (GC 220 with a minimum enzyme activity of 6200 IU/g; data sheet from Genencor) has to be added to hydrolyse the resulted corn silage slurry the dry substance (DS) of the resulting material is determined using the dry cabinet method (described in chapter 3.3.4). Table 3.4.1 shows the enzyme dosages for the different experiments.

For the enzymatic hydrolysis of the pre-treated corn silage, 500g of the pre-treated material is weighed out into stainless steel cans (Edelstahl Dose mit Deckel, 2500ml, 1-1185, NeoLab, D-69123 Heidelberg, Germany) and the pH of the material is corrected to 4.0 to 4.2. Then the temperature is adjusted to 55°C. After reaching the 55°C the cellulase complex GC 220 (Genencor International, Leiden, Netherlands) is added to start the enzymatic hydrolysis. Experiments using

different enzymatic hydrolysis periods of 3, 4, 5, 18, 21, 23, 24, 25 and 120hours respectively are performed (see table 3.4.2).

Pre-treatment temperature [°C]	Dwell period [min]	Dosage of GC 220 [µl/g DS]
150	30	200
150	40	200
150	50	50
150	50	100
150	50	200
160	30	50
160	30	200
160	30	300

Table 3.4.1 GC 220 dosage for the different corn silage and triticale starch SSF experiments

The mashes are stirred at 100 rpm in the stainless steel cans using a Heidolph laboratory stirrer (RZR 2020, Heidolph Instruments GmbH & Co KG, D-91126 Schwabach, Germany) and a propeller stirrer (Roth 0867.1, 50mm, Roth D-76231 Karlsruhe, Germany).

Pre-treatment temperature [°C]	Dwell period [min]	Enzymatic hydrolysis using GC 220 [h]
150	30	3
150	40	3
150	50	3
150	50	4
150	50	23
150	50	24
150	50	25
150	90	5
160	30	18
160	30	19
160	30	21
160	30	120

Table 3.4.2 Hydrolysis periods for using GC 220 for different experiments before the addition of milled triticale grist (<0.5mm)

To reduce the viscosity of the mashes, the corn silage mashes are enzymatically pre-hydrolised. After this enzymatic pre-hydrolysis of the cellulosic material, 150

and 57g, respectively, of triticale grist, milled with a hammer mill using a 0.5 mm sieve, are added. The mash is well homogenized before the pH is adjusted to 3.5 to 4.0. As with the experiments only using starch as substrate, the enzymes Optimash BG and GC 626 (both enzymes Genencor, Leiden, Netherlans) are then added. The dosages of the enzymes are shown in table 3.4.3. To hydrolyse the starch, a temperature of 55°C is maintained for 2 hours. At this temperature the cellulase enzyme complex GC 220 is also still active. Thus, during these 2 hours cellulose is also hydrolysed.

Following this 2 hour treatment, the slurry is cooled down to 30°C and 3g of rehydrated Thermosacc (*Sacchoromyces cerevisiae,* Alltech, Germany) yeast, and the enzymes GC 106 and Stargen 001 (both enzymes Genencor, Leiden, Netherlands) are added (dosage see table 3.4.3). The mash is transferred completely into an Erlenmayer flask for fermentation, which is sealed with an airlock and bung. The Erlenmayer flasks are filled up to a total weight of 500g and put into a water bath of 30°C. At different points in time, ca. 10ml of mash is taken as sample for HPLC analysis. To prepare the samples for HPLC analysis, they are filtered using a plastic syringe and a 0.45µm sterile filter.

Enzyme	Enzyme dosage [ml] 57g Triticale	Enzyme dosage [ml] 140g Triticale
Optimash BG (dilution of 124µl in 25ml)	0.41	1,0
GC 626 (dilution of 620µl in 25ml)	0.41	1,0
GC 106 (dilution of 111µl in 25ml)	0.41	1,0
Stargen 001 (dilution of 1646µl in 25ml)	0.41	1,0

Table 3.4.3 Dosage of amylolitic enzymes for the combined SSF experiments of corn silage and triticale grist

The HPLC analysis is performed using a Bischoff HPLC system which is equipped with a:

- Refractive index detector (ERC 7510, Bischoff, Leonberg Germany)
- RHM monosaccharide column (Phenomenex, Aschaffenburg, Germany)

- Auto sampler (728, Bischoff, Leonberg, Germany)
- Column thermostat (Jetstream 2 PLUS, Bischoff, Leonberg, Germany)

- HPLC conditions:
  - Flow rate 0.6ml/min
  - Mobile phase 0.01n H_2SO_4
  - Column temperature 80°C
  - Detector temperature 35°C
  - Run time 30 min

Calculation of the cellulose hydrolysis rate

To get the proportional ratio of hydrolysed cellulose, the determinable ethanol and remaining concentration of free fermentable sugars in the mash are calculated back to their original concentration of glucan in the raw material. Referring to starch based ethanol yields during the experiments using high dry substance over 30% here a transformation of starch to ethanol only is calculated with a transformation rate of 90% of the theoretical, because of the high and comparable dry substance used during the experiments. Thus all the glucan that is converted to ethanol or hydrolysed to fermentable sugars, less the ethanol that is supposed to be produced from starch, is described as the proportional hydrolysis of cellulose. The calculation is shown in detail in formula 3.4.4.

$$Cell_{hyd}\,[\%] = \frac{\frac{EtOH_{mean}}{0.51} - 0.9 \cdot (EtOH_{Sil} - EtOH_{Trit})}{Gluc_{Cell}} \cdot 100$$

Formula 3.4.4 Percental hydrolysis of cellulose during the process

where:

$Cell_{hyd}$	=	hydrolysed cellulose [%]
$EtOH_{mean}$	=	arithmetric mean of ethanol content in fermented mash [g/l]
0.51	=	theoretical transformation rate of glucose to ethanol during fermentation
0.9	=	90% of starch transformation to ethanol
$EtOH_{Sil}$	=	stoichiometric expected ethanol from starch (Maize silage) [g/kg]

$EtOH_{Trit}$ = stoichiometric expected ethanol from starch (Triticale) [g/kg]

$Gluc_{Cell}$ = maximum glucose from a complete cellulose hydrolysis [g/kg]

100 = factor for the correction to percent

3.5. Different strategies to improve the fermentation of starchy materials

If ethanol is to be produced from renewable materials like starchy and lignocellulosic biomass in one production plant, besides the biomass hydrolysis process, the attention of optimisation also has to be turned on the process that is rampant and optimised as far as possible in industrial distilleries which is the "starch-to-ethanol" process. In industrial scale distilleries ethanol yields of 90 to 95% of theoretical maximum have already been realized (THOMAS and INGLEDEW, 1992). So further improvement of the process can only be reached by decreasing the process duration. Decreasing the process duration makes it possible to produce more ethanol in the same time. But this only makes sense if the ethanol yield does not decrease compared to the 90 to 95% of theory as mentioned above. One strategy to produce more ethanol in a distillery and thereby increase the product (ethanol) time yield is the usage of high-gravity mashes as described by THOMAS and INGLEDEW (1992). But one problem that occurs when high-gravity mashes are fermented is the problem that the fermentation time increases when the dry substance content of the raw material is increased in the mash. This occurs because increasing the sugar concentration in a mash and thereby trying to increase the ethanol concentration in the process retards the growth of the yeast (BEAVEN et al., 1982; CASEY and INGLEDEW, 1986; KALMOKOFF and INGLEDEW, 1985; NAGODAWITHANA and STEINKRAUS, 1976). LUONG (1984) showed that over an ethanol concentration of 112g/l the yeasts have not been to grow any more. So increasing the dry substance content and thereby increasing the potential sugar and ethanol content of a mash could run into problems because the fermentation could eventually stop. But the exact concentration at which the yeast ceases to grow can be influenced by several factors. By altering the nutritional conditions it is possible to increase the survival

of yeasts at high concentrations of ethanol (KOLOTHUMANNIL and INGLEDEW, 1990). Using cereals such as wheat as raw material the mashing procedure liberates nutrients like amino acids from the proteins which are needed for the yeast growth. Some studies showed that it was possible to ferment mashes with high dry substance contents and yield ethanol concentrations of more than 112g/l. Usually such high ethanol concentrations stop the yeasts growth. THOMAS and INGLEDEW (1992) have been able to produce over 20.4% (v/v) of ethanol by starting the fermentation of a “very high gravity” mash containing 300g or more of sugars per litre with an inoculation level of 76 million yeast cells/g mash with the addition of yeast extract as nutrition and with an inoculation level of 750 million yeast cells/g mash without the addition of any nutrient. In another study of KOLOTHUMANNIL and INGLEDEW (1990) the tests yielded 16.9% (v/v) of ethanol from a mash with 35g/l of dissolved solids within 8 days by lowering the fermentation temperature to 20°C. In a third study of JONES and INGLEDEW (1994) it has been possible to produce more than 14 % (v/v) of ethanol from mashes containing 30.3g/100ml and 36.5g/100ml of dissolved solids. The resulting ethanol yield and the fermentation time that has been needed to reach the ethanol yields depended on the fermentation temperatures between 17 and 33°C. The lower the fermentation temperature has been, the longer the fermentation took place. In all three studies the production of ethanol at such high levels has taken at least 72h. Regarding the effectiveness of these experiments, not all tests use the raw material effectively. But, by adding an amino nitrogen source, such as yeast extract or urea, they have been able to reduce the fermentation time and to increase the conversion of the raw material to ethanol.

3.5.1. Addition of nitrogen sources to increase the fermentation speed

Because of the effect that the addition of nitrogen as a nutrient seems to decrease the fermentation time, experiments using urea as a cheap nitrogen source are performed. Additional tests adding the protease GC 106 (Genencor, Leiden, Netherlands) to digest the proteins of the raw material triticale to amino acids and test using thin stillage as a nitrogen source are carried out.

Mashing

To produce the mashes for the experiments 166.304g of Triticale (Lamberto) grist are weighted into a stainless steel mash can and well homogenized with 230ml of tap water (fig. 3.5.1). In the experiments using stillage as the nutrient source, 230g stillage are used instead of the tap water. The weight of 166.304g of triticale grist corresponds to a theoretical ethanol yield of 15%vol. at the end of a complete fermentation. Then the pH has to be corrected to the range of 6.0 to 6.5 considering the buffering of the mash. Now the liquefying enzyme Liquozyme 280L (Novozymes, Bagsvaerd, Danmark) (dosage shown in table 3.5.1) is added and the temperature of the mash is heated to 65°C stirring the mash at 200rpm. The temperature of 65°C is maintained for 30min before the mash is cooled to 52°C. Now the pH has to be corrected to the range of 5.0 to 5.2 and the enzymes SAN Super (Novozymes, Bagsvaerd, Danmark) and Gammaclast 2OP (Gamma Chemie GmbH, Darmstadt, Germany) are added (dosage shown in table 3.5.1). The 52°C are held for a period of 30min before the mash is cooled down to the fermentation temperature of 30°C.

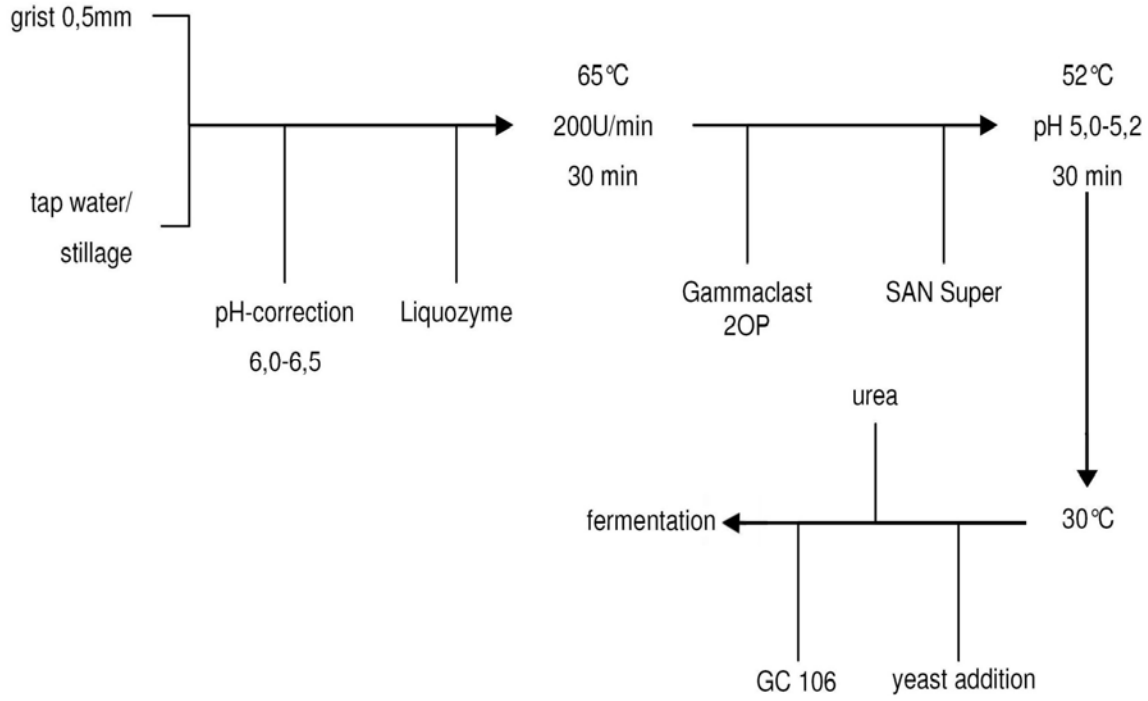


Fig. 3.5.1 Flow chart of the experiments with different fermentation strategies

Name of the enzyme	Dilution in 25ml [ml]	Dosage [ml]
Liquozyme 280L	0.547	1.0
SAN Super 240L	1.367	1.0
Gammaclast 2OP	1.1g	1.0

Table 3.5.1 Dosage of the enzymes for the saccharification of the triticale

Fermentation of the sweet mash

The cooled sweet mash is transferred completely into a 1l-Erlenmeyer flask and 1g of re-wetted *Saccharomyces cerevisiae* (Thermosacc^{TM}, Alltech Germany), a drop of antifoam, the dosage of urea and the protease GC 106 as shown in table 3.5.2 are added.

Experiment	Nitrogen dosage	Dosage of GC 106 [ml]
stillage	used instead of the 230ml of tap water	-
500mg/l urea	500mg/kg urea	-
500mg/l urea and GC 106	500mg/kg urea	0.232ml of a dilution of 1ml in 25ml
1000mg/l urea	1000mg/kg urea	-
1000mg/l urea and GC 106	1000mg/kg urea	0.232ml of a dilution of 1ml in 25ml
no additives	-	-

Table 3.5.2 Dosage of the nitrogen and GC 106 before the fermentation

Before fermentation starts the Erlenmeyer flask is filled up with tap water to a complete mash weight of 500g, sealed with an airlock and bung and put into a water bath tempered at 30°C. From time to time samples are taken to analyse the performance of the fermentation.

To examine the ethanol and sugar concentration during fermentation, a HPLC analysis is performed on a Bischoff HPLC system. Before the HPLC analysis, each sample has to be filtered through a paper filter and the resulting filtrate is filtered a second time using syringes fitted with 0.45µm syringe filters (7-8703, 25mm RC filter, Neo Lab, Heidelberg, Germany).

The HPLC system is equipped with a:

- Refractive index detector (ERC 7510, Bischoff, Leonberg Germany)
- RHM monosaccharide column (Phenomenex, Aschaffenburg, Germany)
- Auto sampler (728, Bischoff, Leonberg, Germany)
- Column thermostat (Jetstream 2 PLUS, Bischoff, Leonberg, Germany)

- HPLC conditions:
  - Flow rate 0.6ml/min
  - Mobile phase 0.01n H_2SO_4
  - Column temperature 50°C
  - Detector temperature 35°C
  - Run time 30 min

3.5.2. The fermentation of various starchy materials at different temperatures and dry substance contents

As already mentioned before, in every distillery process using starchy raw materials the duration of the fermentation is the most important factor that decides how long the total process of ethanol production lasts. On one hand the process has to be as effective as possible. This means that all the fermentable sugar liberated during the hydrolysis process has to be transformed to ethanol. And, on the other hand, the fermentation has to be as fast as possible because in a distillery using the batch fermentation process, every fermenting mash occupies one tank until the fermentation is finished. The sooner this tank can be used for the next mash, the greater the amount of ethanol that can be produced using the same equipment. Thus, a fast fermentation results in a better usage of the tanks and, therefore, in a lower investment in equipment because less tanks have to be built.

The tests described below are performed using different starchy raw materials such as wheat and maize respectively at different dry substance and at different fermentation temperatures. The aim of these tests is to study whether a higher space-time yield can be produced by using high gravity mashes or increasing the temperature (thereby increasing the biocatalytic speed of the yeast cells).

Mashing

To produce the fermentable mash a standardised hydrolysis process is performed. Before the tap water is added, the milled raw material, milled using a 0,5mm sieve, is weighed in. Depending on the DS of the material (table 3.5.3), the correct amount in relation to the content of DS to be contained in the mash is weighed in (table 3.5.4 and 3.5.5). Two different kinds of experiments are performed.

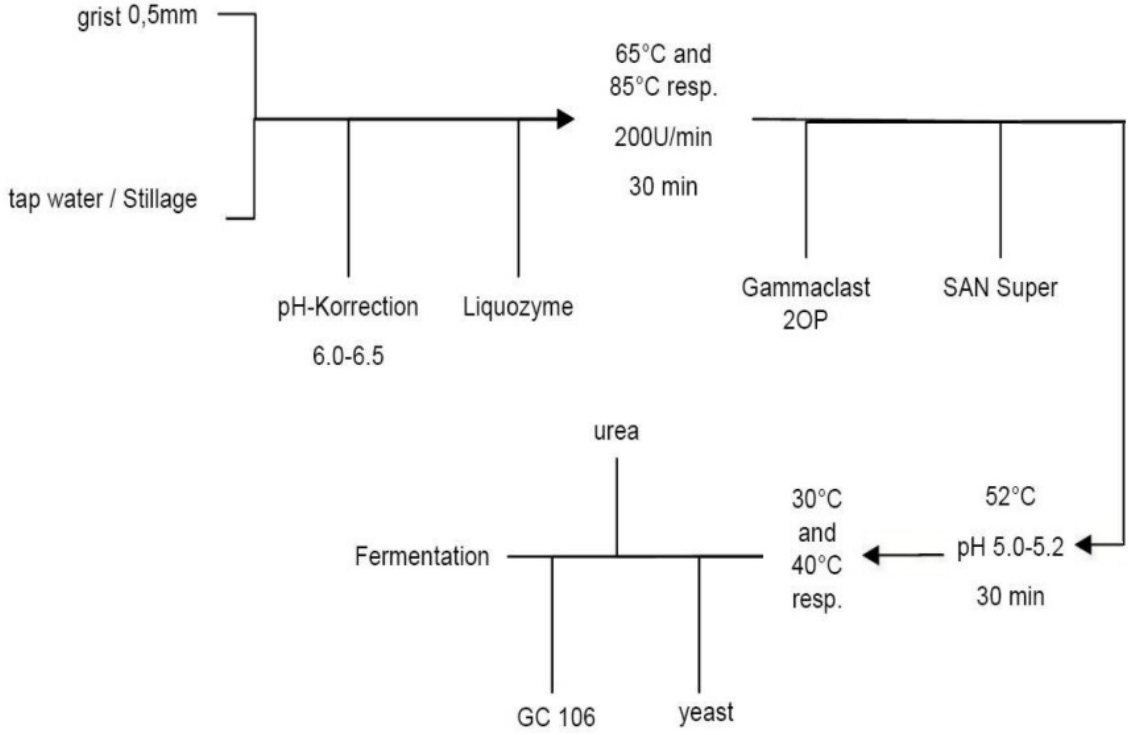


Fig. 3.5.2 Flow chart of the experiments using maize and wheat mashes with different dry substance contents at different temperatures for fermentation

Raw material	DS of the raw material [%]	fermentable substance [%]	Kjeldahl-nitrogen [% of DS]
Wheat	93.10	58.75	15.06
Maize	90.08	64.87	10.51

Table 3.5.3 The DS of the different raw materials

The one kind of experiments has a total mash weight of 250g and the others a total mash weight of 300g. After the water has been added and the mash mixed homogenously, the pH has to be corrected to pH 6.0 to 6.5. Then the amount of the liquefying enzyme Liquozyme 280L (Novozymes, Bagsvaerd, Denmark) is added. As described by the manufacturer it is adjusted to the starch content of the

mashes (200ml/t starch). Then the mash is heated to 65°C for the wheat mashes and 85°C for the maize mashes respectively for 30min (fig. 3.5.2).
After this time period the mash is cooled down to 52°C and the pH has to be corrected between 5.0 to 5.2. The Glucoamylase SAN-Super (Novozymes, Bagsvaerd, Denmark) and the saccharification enzyme complex Gammaclast 2OP (Gamma Chemie GmbH, Darmstadt, Germany) are added.

DS content in the mash [%]	Weight-in for the 250g mashes [g]	Weight-in for the 300g mashes [g]
20	53.71	64.45
25	67.13	80.56
30	80.56	96.67
35	93.98	112.78

Table 3.5.4 The weight in of wheat grist for the experiments

Their dosages are (as described by the manufacturers: SAN-Super 500ml/t starch and Gammaclast 2OP 400g/t starch) adapted to the starch content of the mashes. Now the saccharification of the starchy material is performed for 30min.

DS content in the mash [%]	Weight-in for the 250g mashes [g]
20	55.51
25	69.38
30	83.26
35	97.14

Table 3.5.5 The weight in of maize grist for the experiments

Fermentation of the sweet mash

After hydrolysis of the starch at the end of the mashing the mash is cooled down to fermentation temperature and 1.0g of the re-wetted yeast Thermosacc^{TM} and Superstart (both *Saccharomyces cerevisiae* strains kindly donated by Alltech, Sarney, Ireland) respectively are added to start the fermentation. The fermentations are performed for 72h in a water bath of 30°C and 40°C respectively or using an Inforce shaker mixing at 80rpm.

3.5.3. The influence of dry substance (DS) on the fermentation yield using the stargen™ process

Another strategy to optimise the starch to ethanol process is to save electrical and thermal energy. As described before, using the stargen™ enzyme system no heating over gelatinising temperature is needed to liberate fermentable sugars from starchy materials. Therefore, a lot of energy can be saved during the mashing process because of the lower viscosities and lower thermal energy demand (lower temperatures). But normally not only lot energy is used during mashing. The distillation process also has a huge energy demand. And the energy requirement of a distillation column is dependent on the ethanol content of the mash to be distilled. Fig. 3.5.3 shows the energy requirement of a single distillation unit for the production of ethanol with a concentration of 94.5% (w/w). Mashes with lower ethanol concentrations need a lot more energy to be concentrated to a high ethanol concentration. The distillation of mashes only becomes profitable starting at ca. 4% (w/w) of ethanol. The higher the ethanol concentration of the mash becomes, the more the energy requirement per kg yielded ethanol decreases.

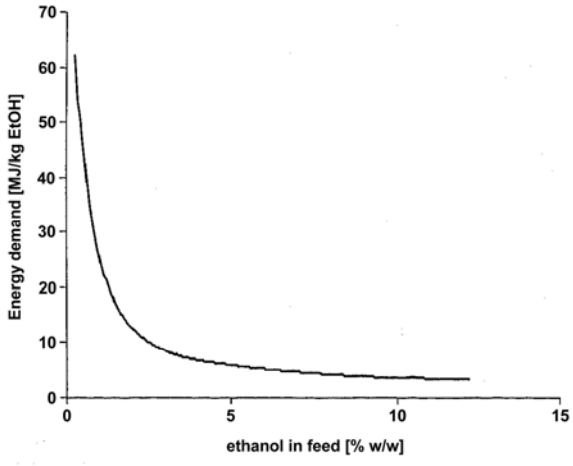


Fig. 3.5.3 Energy demand in a single distillation unit fort he production of 94.5% (w/w) of ethanol from diluted ethanol feed (source: ZACCHI and AXELSON, 1989 modified)

So if energy is to be saved during distillation, the mashes should contain as much ethanol as possible. But if high ethanol yields are to be produced, high concentrations of fermentable sugars also have to be available in the fermenting mash. And high concentrations of fermentable sugars demand the use of high weight-in of cereals and, therefore, high dry substance contents. In this test the influence of the dry substance during the SSF process using "stargen™" enzymes

is observed because only producing high ethanol concentrations does not result in a profitable ethanol process. Also the profit originated from the cereals input has to be taken in consideration. A good ethanol process uses the cereal as completely as possible. In these experiments the yield of ethanol resulting from the cereals is to be examined using the new enzyme system.

For each test milled triticale with a particle size < 0.5mm is weighed into a stainless steel mash can (shown in fig. 3.5.4 a; Edelstahl Dose mit Deckel, 2500ml, 1-1185, NeoLab, D-69123 Heidelberg, Deutschland). Additional 200ml deionised water is added. Then the slurry is mixed until it is homogeneous and the pH of the slurry is corrected to between 3.5 and 4.0 using 0.1n H_2SO_4. Now the temperature is heated up to 55°C and the enzymes Optimash BG and GC 626 (both enzymes Genencor, Leiden, Netherlands) are added (dosage see table 3.5.6). The mashes were stirred at 100 rpm using a Heidolph laboratory stirrer (RZR 2020, Heidolph Instruments GmbH & Co KG, D-91126 Schwabach, Germany) and a propeller stirrer (Roth 0867.1, 50mm, Roth D-76231 Karlsruhe, Germany). The temperature of 55°C is maintained for a dwell period of 2h. After this the slurry is cooled down to 30°C and 3g rehydrated Thermosacc (*Sacchoromyces cerevisiae,* Alltech, Germany) yeast, and the enzymes GC 106 and Stargen 001 (both enzymes Genencor, Leiden, Netherlands) are added (dosage see table 3.5.6).

Experiment	Triticale weight in [g]	Dry substance	Optimash BG (dilution of 124µl in 25ml) corresponding to 61,08ml/t starch	GC 626 (dilution of 620µl in 25ml) corresponding to 305ml/t starch	GC 106 (dilution of 111µl in 25ml) corresponding to 55ml/t starch	Stargen 001 (dilution of 1646µl in 25ml) corresponding to 810ml/t starch
1	140	25.2	1.0ml	1.0ml	1.0ml	1.0ml
2	175	31.5	1.22ml	1.22ml	1.22ml	1.22ml
3	205	36.9	1.46ml	1.46ml	1.46ml	1.46ml

Table 3.5.6 Dosage of enzymes for the different experiments

To ferment the mash, it is transferred completely into an Erlenmayer flask which is sealed with an airlock and bung. The Erlenmayer flasks are filled up to a total weight of 500g and put into a tempered water bath of 30°C. At different points of time ca. 10ml mash is taken as a sample for HPLC analysis. To prepare the

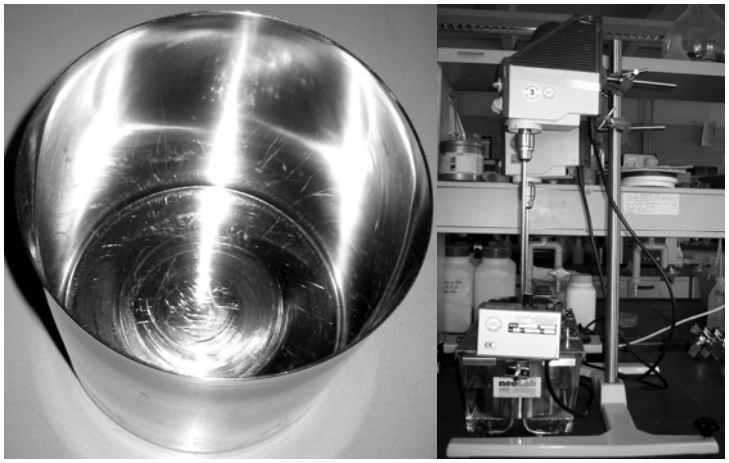
samples for HPLC analysis, each sample is filtered using a plastic syringe and a 0.45µm sterile filter.

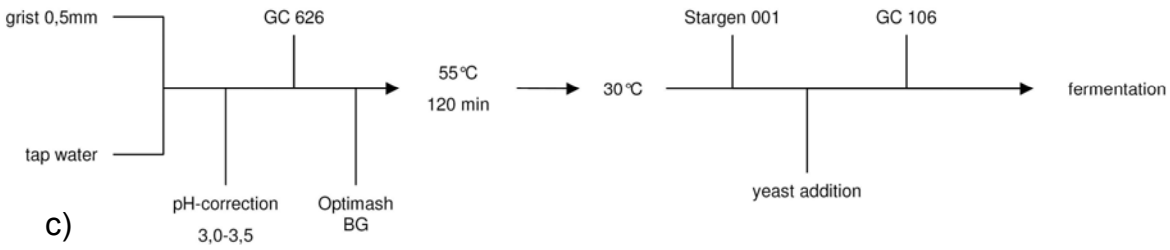
The HPLC analysis is performed using a Bischoff HPLC system which is equipped with a:

- Refractive index detector (ERC 7510, Bischoff, Leonberg Germany)
- RHM monosaccharide column (Phenomenex, Aschaffenburg, Germany)
- Auto sampler (728, Bischoff, Leonberg, Germany)
- Column thermostat (Jetstream 2 PLUS, Bischoff, Leonberg, Germany)

- HPLC conditions:
  - Flow rate 0.6ml/min
  - Mobile phase 0.01n H_2SO_4
  - Column temperature 50°C
  - Detector temperature 35°C
  - Run time 30 min

a) b)





c)

Fig. 3.5.4 a) stainless steel can for hydrolysis experiments. b) Experiment arrangement for enzymatic hydrolysis. c) Flow chart of the experiments using the stargen™ enzymes

3.6. Experiments using residues of the ethanol process

In order to complete a renewable process, also residues of the ethanol process are to be used sensefully. One possibility is the production of bio-methane.

3.6.1. Bio-methane analysis

The analysis of bio-methane in the resulting stillage is performed according to the Hohenheimer biogas test (HBT) at the Landesanstalt für landwirtschaftliches Maschinen- und Bauwesen of the Universität Hohenheim in Stuttgart.

The basis of this method is the so called “Hohenheimer Futterwerttest”, that indicates the energetical feed value of fodder for ruminants. The test is performed using glass syringes (flask samplers) as shown in fig. 3.6.1, with a volume of 100ml and a 1/1 grading as well as a capillary at the end for the gas analysis as fermenter. The capillary is connected to a gastight tube, which can be sealed with a clip. An inert sealant is used to seal the gap between the syringe and the stopper. About 60 of this closed glass syringes are fitted into a motor-driven rotor that rotates for mixing the samples. The complete rotor is integrated in an incubator so that the correct fermentation temperature can be set.

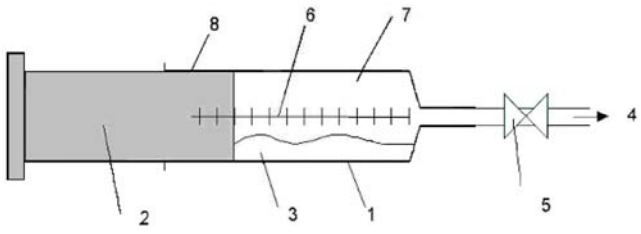


Fig. 3.6.1 glass syringe for the HBT method (1 flask sampler glass syringe; 2 stopper; 3 fermentation substrate; 4 capillary for methane analysis; 5 clip; 6 grading 1/1; 7 head space for methane; 8 lubricant and sealant)

To perform a HBT inoculated substrate such as digested liquid manure from an agricultural biogas plant or other inoculated substrate containing all the bacteria needed for the biogas fermentation is required. The ideal ratio of inoculated liquid manure to substrate lies between 2-3 : 1 (HELFRICH and OECHSNER, 2003). Depending on its dry matter content, a representative sample of the test material is taken. First the dry substance (DS), organic substance (OS) and ash content of the sample is determined. Afterwards the sample is gently dried in a dry cabinet at 50-60°C for 48h and milled using a cutting mill so that it passes through a one millimetre sieve. For the experiment CO_2 is added to the inoculated liquid manure while it is roughly sieved and stirred continuously in a bath with regulated temperature. Ca. 30ml of the sieved inoculated manure is put into a glass syringe and its quantity is weighed to a precision of 1/100 gram. Then 500mg (precision of

1/1000 gram) of test substrate are added. Using the stopper all residual air has to be evacuated from the glass syringe to make sure that the fermentation broth is anaerobic. The glass syringe is now sealed gas-tight. Each test is performed in triplicate, at least, and additionally at least three tests with pure inoculated liquid manure are also performed. These three pure manure tests form the blank value. The sealed glass syringes are now put into the rotor and the HBT is performed until almost all the substrate has been transformed to gas. The test is stopped when only 1% of the already yielded gas is generated within one day (HELFFRICH and OECHSNER, 2003). To evaluate how much methane is yielded, the resulting volume of gas is determined using the grade of the glass syringe. Additionally, the content of methane in the gas is measured. Knowing both these data, it is easy to calculate the amount of yielded methane. The calculated result is then based on dry substance or organic dry substance of the test substrate.

4. Results

4.1. Lignocellulosic biomass

4.1.1. The influence of the pre-treatment conditions on the enzymatic hydrolysis of corn silage using direct heating for pre-treatment

Different pre-treatment temperatures should produce different results regarding the accessibility of the corn silage. Because of this different dwell periods for the different temperatures have to be tested. For every raw material different optimum conditions exist at which the yield from the glucan is at a maximum after enzymatic hydrolysis. The raw material corn silage that is used in these experiments consists of different glucan sources. For corn silage starch and cellulose are two sources that are built of glucose.

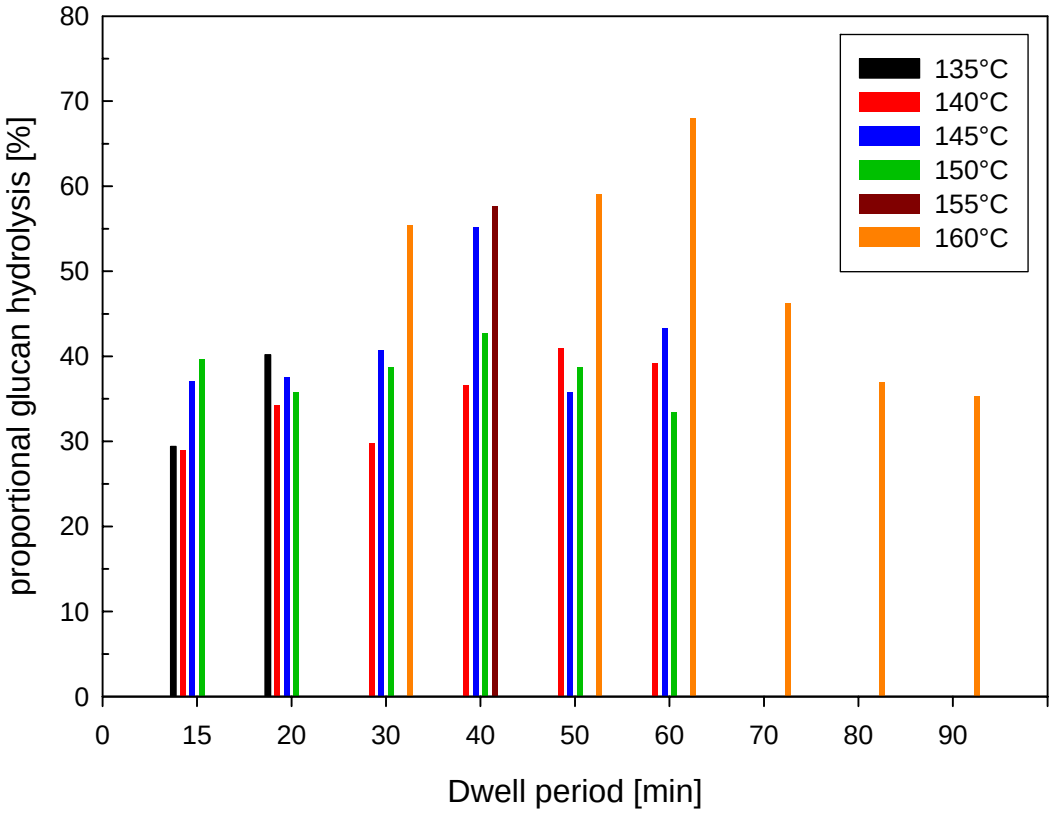


Fig. 4.1.1 The proportional glucan hydrolysis of direct heated maize silage after 48h of enzymatic hydrolysis based on the theoretical glucan content (every test series, n=2)

But what makes the pre-treatment of this material difficult, is that starch and the cellulose which enzymatically can be hydrolysed to fermentable glucose need different pre-treatment conditions to be hydrolysable. These tests should try to detect the ideal pre-treatment process that offers an optimal enzymatic glucan hydrolysis and, therefore, for an optimal glucan hydrolysis yield before a potential fermentation.

As can be seen in fig. 4.1.1, increasing the dwell period from 15 to 20min at a temperature of 135°C (black columns) resulted in an increase of 10% from ca. 30% up to 40% regarding the accessibility of the biomass. Increasing the temperature from 135 to 140°C (red columns) generates a negative effect on the digestibility of the biomass for comparable dwell periods. If the material is pre-treated at 140°C for 30min the digestibility, which at first increased with increased dwell period, in contrast to the prospects decreased. But further increasing the pre-treatment duration, result to a rise of the biodegradability up to 40% (during the 48h hydrolysis) for a pre-treatment for 50min. Longer holding times >50min for 140°C produce a decrease in the digestibility. A 40min pre-treatment of the corn silage at 145°C (blue columns) results in a maximum glucan hydrolysis yield for the temperature of 145°C. These process parameters make it possible to hydrolyse ca. 55% of the total glucan within 48h. Shorter or longer pre-treatment times, such as 15 to 30min and 50 to 60min respectively, lead to lower hydrolysis yields. Further increases in the pre-treatment temperature to 150°C (green columns) for a 15min dwell period results in a digestibility of ca. 40% what is comparable to the results of 130°C for 20min and 145°C for 30min respectively. Increasing the pre-treatment duration at 150°C to 20 and 30min respectively results in a decrease in digestibility. After a slight increase in biodegradability for a dwell period of 40min up to ca. 43%, further increasing the holding time produces a decrease. At these conditions perhaps the cellulosic biomass was more accessible due to the higher temperature. But the higher temperature simultaneously caused a higher loss of sugars originating from starch because of maillard products formed from proteins and reducing sugars. Thus, the maximum glucan hydrolysis that can be yielded decreased due to the increase of the temperature. At 155°C (dark pink columns) only one pre-treatment time (40min) is tested. For these parameters ca. 58% glucane hydrolysis could be yielded after 48h enzymatical hydrolysis. For the highest temperature of 160°C (orange

columns) a maximum of glucan hydrolysis, in the range between 30 to 60min of pre-treatment duration, is resulted. Even for 30min of pre-treatment time a 55% hydrolysis of the glucan is yielded in the enzymatic hydrolysis. Increasing the pre-treatment duration to 50 and 60min respectively results in a 60 to 70% hydrolysis of the biomass to glucose. This was the maximum hydrolysis yield that could be obtained for all experiments that are performed. Longer hydrolysis times than 60min at 160°C result in a decrease in the hydrolysis yield down to 30%.

Regarding all the results that are determined during the different pre-hydrolysis tests the conditions using 160°C and a dwell period of 50 to 60min seem to be the best parameters for a successful enzymatic corn silage hydrolysis. By using these parameters it is possible to hydrolyse up to 70% of the glucan within 48h. But one problem that still has to be mentioned is the relatively low DS of ca. 9.5% that only can result in maximum ca. 25.5g ethanol per litre. Using such mashes it is impossible to have an economic distillation step in a distillery because of the huge amount of energy that is needed to distil such mashes.

4.1.2. The influence of dispersing the raw material before the direct heated pre-treatment on its enzymatic digestibility

Unlike to the experiments described before, here the corn silage is dispersed using a Becomix DH 1220 before the pre-treatment. By dispersing the raw material the particle size is clearly smaller than for the tests that used the corn silage without any size reduction. After the pre-treatment, as a result of the dispersing and heating of the corn sillage the fibres are smaller in size than 1mm. Regarding the results of the comparison of mashes that used dispersed and no size reduced material (fig. 4.1.2), it can be seen that the mashes that were processed using dispersed material, after 48h of enzymatic hydrolysis, produced a significantly higher glucan hydrolysis than the experiments that used no further size reduction.

For the pre-treatment conditions of 160°C for 50min an increase in biodegradability of ca. 25% from 38.12 % up to 64.93% is caused by dispersing the corn silage. For the pre-treatment at 160°C for 30min the tests without size reduction yielded in a ca. 55% glucan hydrolysis. In contrast, the test at the same conditions using dispersed raw material resulted in a increased digestibility of ca. 12% up to 67.01%. Additionally, it is easier to pump the material into the jetcooker

and to mix the slurry during the hydrolysis process. If the complete hydrolysis process of the biomass is regarded instead of only the hydrolysis time of 48h, a clear advantage of the size reduction, realised with dispersing the material, can be pointed out.

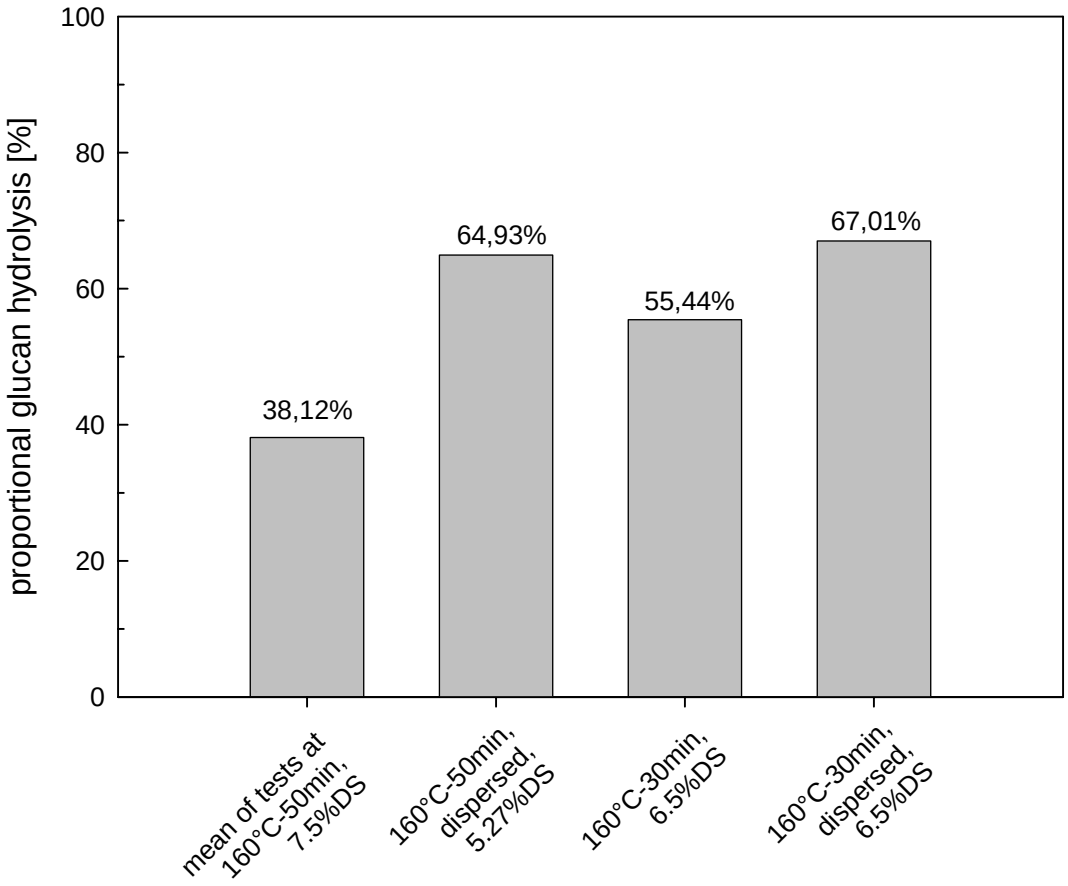


Fig. 4.1.2 The proportional glucan hydrolysis after 48h enzymatic hydrolysis of direct heated dispersed corn silage that was pre-treatment at 160°C (every test series, n=2)

In fig 4.1.3 this advantage is shown. The experiments that are carried out using 160°C and a 50min heating time without any further size reduction result in a glucan hydrolysis of ca. 40% after 48h. But using dispersed corn silage for this pre-treatment conditions ca. 40% hydrolysis is obtained after ca. 20h. With these experiments, it is possible to hydrolyse 67% of the glucan within 48h. Comparing the results obtained after a 50min and 30min of dwell period respectively using the dispersed raw material, nearly the same glucan hydrolysis is obtained after 48h. And in contrast to the tests using a holding time of 50min for pre-treatment, the

hydrolysis progress at the start of the hydrolysis tests increases with a higher gradient. After ca. 70-80h hydrolysis time, both tests using dispersed material produced a maximum yield of ca. 75-80% glucan hydrolysis. One fact that could be ascertained is the fact that after dispersing the biomass even dwell periods of 30min could be enough to make the material accessible for a fast enzymatic hydrolysis.

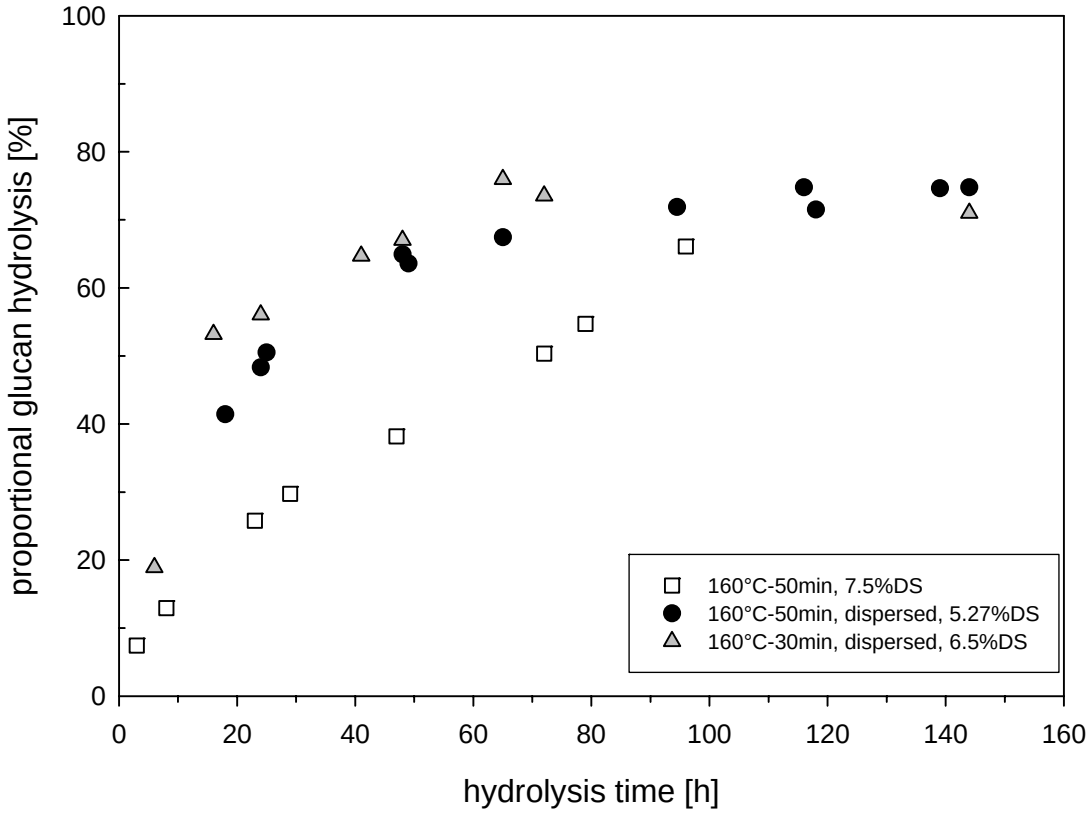


Fig. 4.1.3 Progress of the hydrolysis of dispersed corn silage mashes after direct pre-treatment (every test series, n=2)

The tests that are performed using a 30min dwell period result in the fastest glucan hydrolysis and a 75% hydrolysis already after 70h. The tests that are made using 50min as the dwell period took a bit longer and produced simillar results after ca. 80h. For both experiments longer hydrolysis times did not produce further increase in glucan hydrolysis and therefore, could been stopped after a 70-80h enzymatic hydrolysis.

In contrast to the results with dispersed material, if the material is not dispersed, the hydrolysis of the biomass takes more time to reach similar results. As can be seen in fig. 4.1.3, at least more than 100h of enzymatic hydrolysis would be

needed to achieve ca. 75% of the total glucan hydrolysis which is yielded using the dispersed material.

4.1.3. The influence of the pre-treatment conditions on the enzymatic hydrolysis of dispersed corn silage using indirect heating for pre-treatment

Here the dispersed corn silage is heated indirectly in a different jet cooker than for all the other experiments described before. This jet cooker heats the material using a heating jacket to increase the temperature of the medium to be heated. Heating the material using the indirect heating strategy does not cause any dilution of the material. Consequently, the dry substance content that is inserted into the jet cooker approximately results after the pre-treatment. Probably during the steam explosion some water can be lost due to evaporation out of the reception vessel when the material cools down after pre-treatment.

Figs. 4.1.4 A, B and C show the results of some experiments that were performed. During the first 24h of hydrolysis almost all experiments result in a 30-40% glucan hydrolysis. The only both experiments that did not produce these results were the experiments that are performed at 150°C for 90min and 120min respectively. This may be due to the long heating at 150°C and, therefore, a very strong effect on the starch and liberated sugars that are contained in the biomass. Maybe at these conditions the starch builds maillard complexes with the proteins that are contained in the silage. This effect results in a loss of degradable starch that could not be used for the process any more. Additionally, liberated sugars might be degraded to sugar degradation products as furfurals. In the phase between 24 and 48 hours all experiments result in an increase of 5-10% in their glucan hydrolysis. The only experiment that resulted in a decrease is the experiment using 155°C and a dwell period of 30min (fig. 4.1.4 C, white filled triangles). After 24h this test resulted in the highest yield of ca. 47%, but as the process continued it seemed that the enzymes no longer worked. And, regarding the end result of this test, after 72h, no further glucan could be digested.

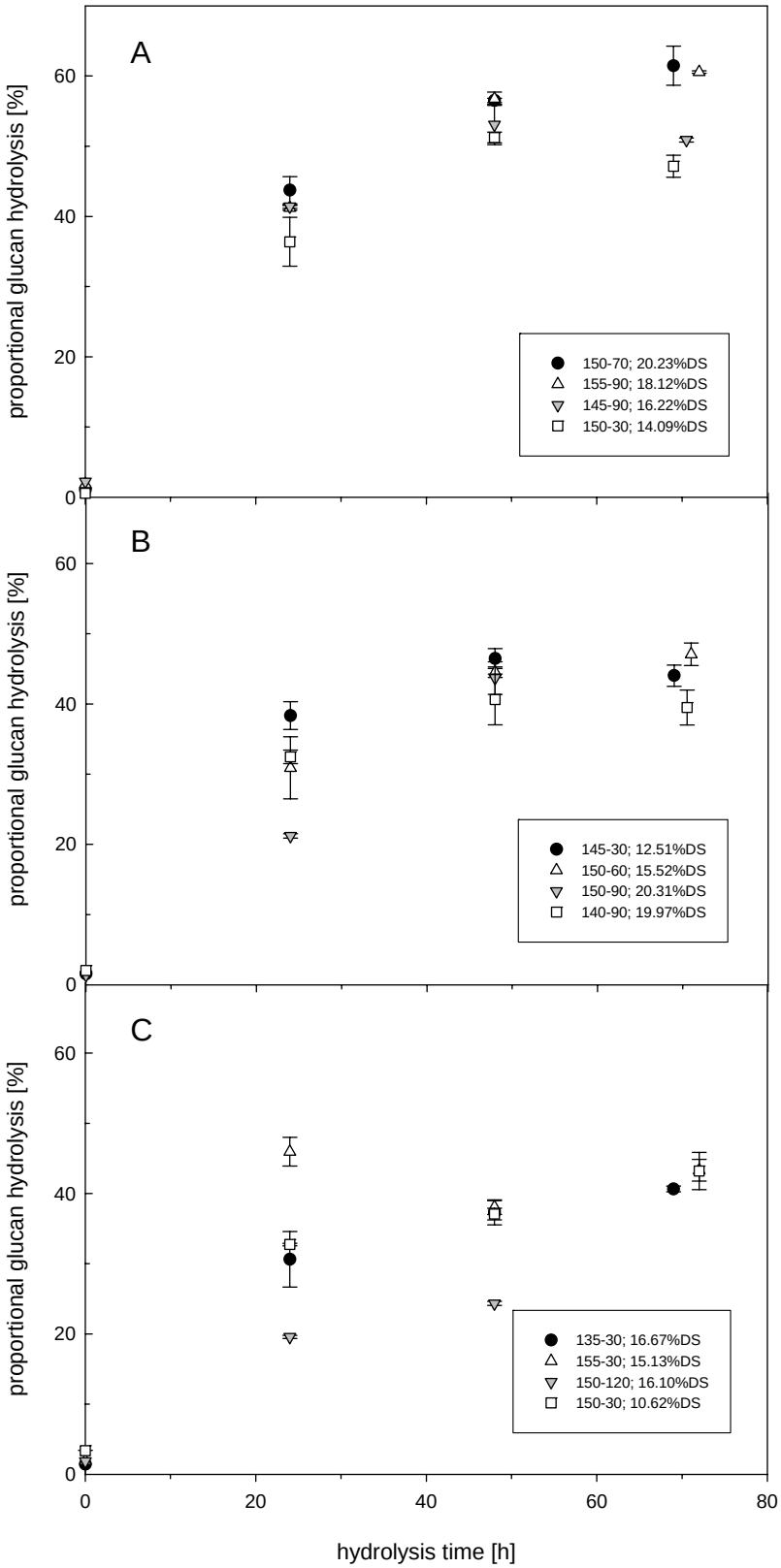


Fig. 4.1.4 Influence of the pre-treatment method using indirect heating on dispersed corn silage (every test series, n=2)

Thus, it would seem that the maximum possible glucan yield using enzymatic hydrolysis was reached after 24h. The other tests both in fig. 4.1.4 C (150°C-30min, 10.62% DS and 135°C-30, 16.67% DS) show slight increases of ca. 10% in the glucan yield when the enzymatic hydrolysis time is increased to 72h. At the end of the given time period the curves for both experiments seem to flatten, so that by further increasing the hydrolysis time no further big increase in hydrolysis yield could be expected. Similarly, the results of the experiments shown in fig. 4.1.4 B an increase of 5-10% can also be yielded during the phase between 24 and 48h. But in the phase between 48 and 72h the curves tend to flatten and finally result in a glucan hydrolysis of ca. 40-45%. The highest results that could be yielded were obtained at 145°C with a relatively long pre-treatment duration of 90min, at 150°C using a dwell period of 30min, 150°C and 70min of heating time and 155°C and 90min of pre-treatment respectively (shown in fig. 4.1.4 A). Here ca. 35-45% of glucan could be hydrolysed in the first 24h for all tests. In the second phase between 24 and 48h, up to ca. 50 to 56% of glucan hydrolysis is obtained. In the case of the experiments that are performed at 150°C and a 30min dwell period and 145°C with a 90min dwell period respectively, the maximum yield is reached at this stage of the process. Further process duration did not produce a further glucan hydrolysis. Only the tests at 150°C (70min) and 155°C (90min) respectively resulted in a further glucan hydrolysis using the longer hydrolysis duration. After 72h both experiments hydrolysed ca. 60% of the glucan to fermentable sugars. Those results are ca. 10% lower than the results that could be yielded using direct heating on dispersed biomass at 160°C. But in contrast to these higher results, the DS that is used in the tests that are described here is much higher. Here 20.23% and18.12% of DS as opposed to 5.27% and 6.5% of DS are used. So the results for the indirect heated corn silage seem to be very good compared to the results that are obtained using direct heating.

In fig. 4.1.5 the maximum theoretical ethanol yields that could be obtained if all the mashes are fermented after a 72h hydrolysis, are shown (theoretical ethanol yield 0.51g ethanol/g glucose). As expected, similar to the results above, the experiments with the highest glucan hydrolysis also would produce in the highest ethanol yield in g/l. Although the experiments using 150°C (70min) and 155°C (90min) nearly had the same glucose content after 72h of hydrolysis and thereby would yield in about the same ethanol yield in g/l the experiment at 155°C (90min)

theoretically yield in a higher ethanol yield per 100kg of DS. Regarding the ethanol yield per 100kg of DS almost all tests can yield in minimum14-16 l of ethanol. The only test that would not yield such an ethanol yield is the experiment at 150°C (120min).

This experiment only can result in maximum 8.45l of ethanol per 100kg DS. This result shows that heating the raw material too long results in a big loss in sugars and thereby, in a big loss of potential ethanol. Taking all the results of the tests that are made into consideration, if a short dwell period should be used, the tests at 150°C heating the biomass for 30 min seems to be the best combination regarding the ethanol yield per 100kg of DS.

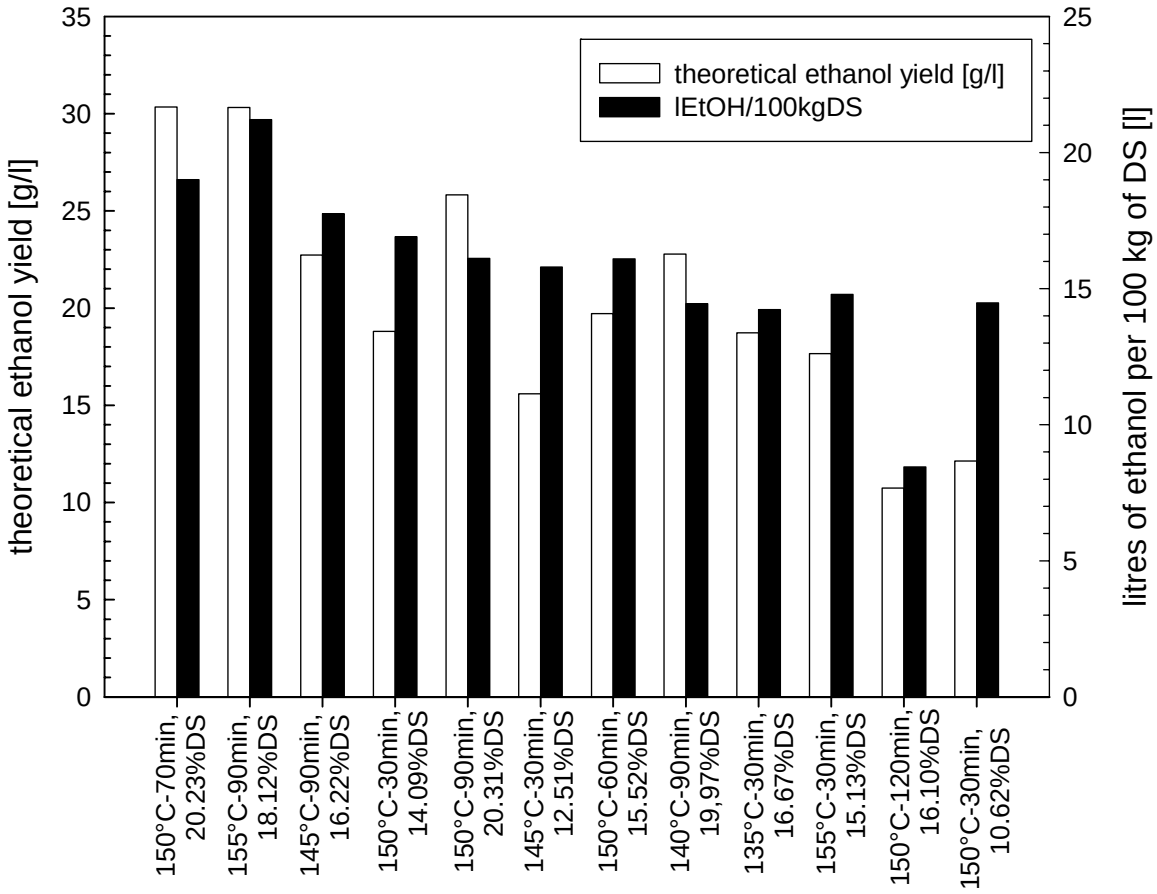


Fig. 4.1.5 Theoretical ethanol yield and ethanol yield per 100kg DS when mash would be fermented (every test series, n=2)

Higher temperatures or longer dwell periods may cause higher energy use during pre-treatment. Furthermore higher and lower temperatures respectively that also used a heating duration of 30min only can result in a lower ethanol yield per 100kg DS.

4.1.4. The optimization of the enzyme dosage using GC 220 for enzymatic hydrolysis of corn silage

The commercially available cellulosic enzyme GC 220 (Genencor, Leiden, Netherlands) was compared to the results that are obtained using GC 880 (Genencor, Leiden, Netherlands) because the samples of GC 880 that were kindly supplied by Genencor ran out. So a new enzyme that was available on the market has to be tested for its effectiveness on pre-treated biomass. For further experiments nearly the same glucan yield should be obtained. However, because the correct enzyme dosage to reach this is not known, tests using different enzyme dosages are performed to get an idea, how much enzyme has to be added for a comparable hydrolysis of the biomass as in the test before. Dispersed corn silage that has been pre-treated at 150°C for 60min is used as substrate. This is in fact not the best biomass for a complete enzymatic hydrolysis. But by using such a relatively suboptimal pre-treated material, the effectiveness of the cellulase complex regarding glucan and hemicellulose hydrolysis can be seen. Fig. 4.1.6 shows the results of the biomass digestion. To compare the hydrolysis process to the tests processed using 180µl GC 880/gDS the progress of such an experiment is also shown. Fig. 4.1.6 A shows the progress of the glucan hydrolysis during the process. At the beginning of the experiments the glucan hydrolysis using GC 220 for all experiment is slightly faster. In the first 24h ca. 30-33% of the glucan could be hydrolysed using GC 220. By way of comparison, using GC 880 results a 27% glucan hydrolysis. This may be because, when using GC 220, the xylan is hydrolysed faster during the first 24h (fig. 4.1.6 B). Compared to the usage of GC 220, using GC 880 resulted in a much faster arabinan hydrolysis. Already after ca. 24h nearly the complete arabinose is liberated. Here using GC 880 seems to hydrolyse the arabinan first and then the xylan. When GC 880 is used for a hydrolysis time of ca. 48h, the hydrolysis of the xylan seemed to stop (fig. 4.1.6 B). Where as using GC 220 resulted in a further slight increase of xylan hydrolysis even during the phase between 48 and 116h. In almost the same manner, depending on the dosage of the GC 220 enzyme, the arabinan yield (fig. 4.1.6 C) increased if the hydrolysis time is increased. But maximum 87% of arabinan was hydrolysed using the highest dosage of 300µl GC 220/gDS.

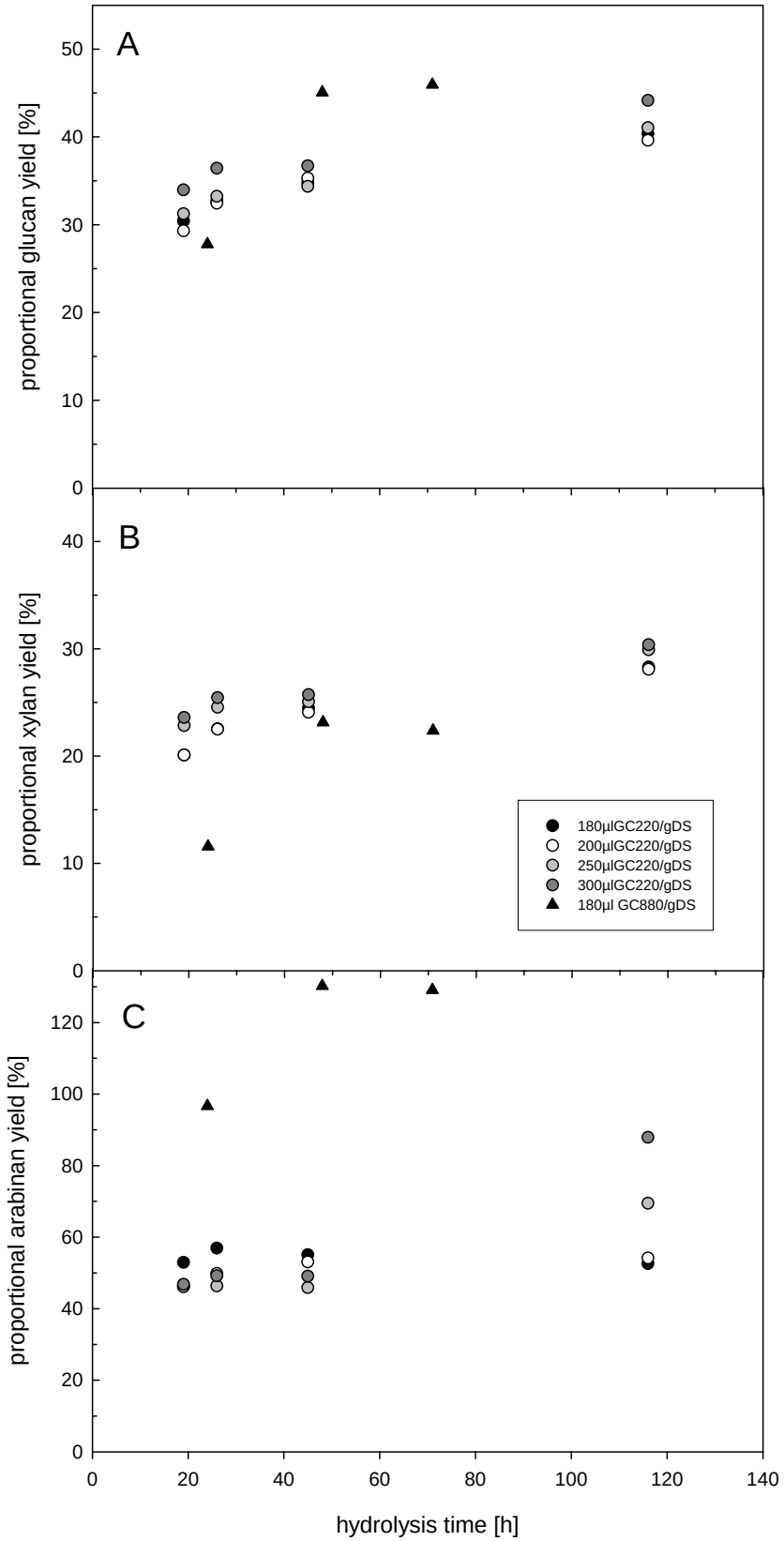


Fig. 4.1.6 Optimization of the enzyme dosage using GC 220 for enzymatic hydrolysis of dispersed maize silage after pre-treatment at 150°C for 60min (every test series, n=2)

If the dosage of the enzyme is reduced, the arabinan hydrolysis decreases to ca. 57% (180-20µlnGC220/g DS) and ca. 75% (250µl GC 220/g DS) respectively. Regarding the xylan hydrolysis for all dosages of GC 220, the yield was almost the same for all experiments. Ca. 25% of the xylan could be hydrolysed during the first 24h. If the hydrolysis time is increased up to 120h a ca. 30% xylan hydrolysis is yielded. The high hydrolysis yield of the arabinan in the first 24h combined with almost the same xylan hydrolysis after 48h of hydrolysis time (compared to the tests using GC 220), using the enzyme complex GC 880, made it possible for more glucan to be digested during the first 72 h of hydrolysis. But after 119h of hydrolysis, the usage of GC 220 also reached the level of glucan hydrolysis that could be reached using GC 880 for 72h. Here between 40 to 45% could be digested, too. Although the usage of 300µl GC 220/g DS results in the highest hydrolysis rate of 45%, the clearly higher enzyme dosage of 300µl GC 220/g DS compared to 200µl GC 220/g DS would produce much higher enzyme costs in a distillery process. Consequently, because of the relatively low loss compared with the relatively large increase in costs that would be produced using the higher enzyme dosage, the dosage of 200µlGC 220/g DS was used for the following experiments.

4.1.5. The influence of different pre-hydrolysis times on the SSF process

In these experiments the influence a pre-hydrolysis before a SSF process is to be examined. To do this corn silage that is pre-treated at 160°C for 30min and at 150°C for 50min respectively is used. To decide if there is an influence of the enzymatic pre-hydrolysis at 55°C and pH 4.0 to 4.2 (using 200µl GC 220/g DS of corn silage), different enzymatic pre-hydrolysis times are performed for the pre-treated material at 150°C for 50min. As can be seen in fig. 4.1.7, the 4h of pre-hydrolysis time at 55°C and, thus also a shorter total hydrolysis time resulted in a faster ethanol building than the other experiments. This is due to the earlier start of the SSF process. Comparable to this, the tests using an enzymatic pre-hydrolysis for 21h and 24 h and material pre-treated at 150°C for 50min, also result in a faster ethanol production in the phase between 40 and 90h of total hydrolysis time. So because of the longer pre-hydrolysis on one hand more glucan (ca. 25-30%)

already is hydrolysed (fig. 4.1.7 B) before yeast addition, but on the other hand no advantage regarding a better biomass hydrolysis and ethanol production respectively is achieved. In contrast to the results already discussed, the experiments using material which was pre-treated at 160°C for 30min on one side resulted in comparable glucan hydrolysis at the end of the tests, but on the other hand had a much longer lag phase. This may be due to the formation of more inhibiting substances such as maillard products from the starch of the corn silage during this pre-treatment resulting in a longer accommodation time for the yeast before it can work optimally. This lag of the higher pre-treatment temperature nearly lasts until 50h of total hydrolysis time. From this point on, when ca. 40% of the glucan have been hydrolysed, the process of the pre-treated material at 160°C catches up. Already after 20h 7% of the expected ethanol is produced from the mash of corn silage pre-treated at 150°C for 50min with a pre-hydrolysis of 4h During the next step of fermentation (20-50h) a great increase in ethanol from 7% up to ca. 40% of theory is resulted. As the total hydrolysis time increases further, for all experiments the production of ethanol (fig. 4.1.7 A) and glucan hydrolysis (fig. 4.1.7 B) respectively still increases. After 162h of total hydrolysis time ca. 80% of the theoretically attainable ethanol is yielded for all experiments. After such a long hydrolysis time the different pre-treatment methods result in almost the same ethanol production. The experiments using 160°C as pre-treatment temperature were performed until ca. 186h of total hydrolysis time are reached. For the pre-treatment at 160°C the final ethanol yield is ca. 83-85% of the theoretical value and the glucan hydrolysis is 85-87%.With the other experiments, which are pre-treated at 150°C, the final ethanol yield at 162h of total hydrolysis time is ca. 77-80% ethanol and 78-82% hydrolysed glucan (fig. 4.1.7 B). The results of the 160°C pre-treatment experiments are comparable to results that could be reached processing only starchy materials at a DS of 30% and therefore, were very good results.

To get the proportional ratio of hydrolysed cellulose (shown in fig. 4.1.8), the resulted ethanol and remaining concentration of free fermentable sugars in the mash were calculated back to their original concentration of glucan in the raw material.

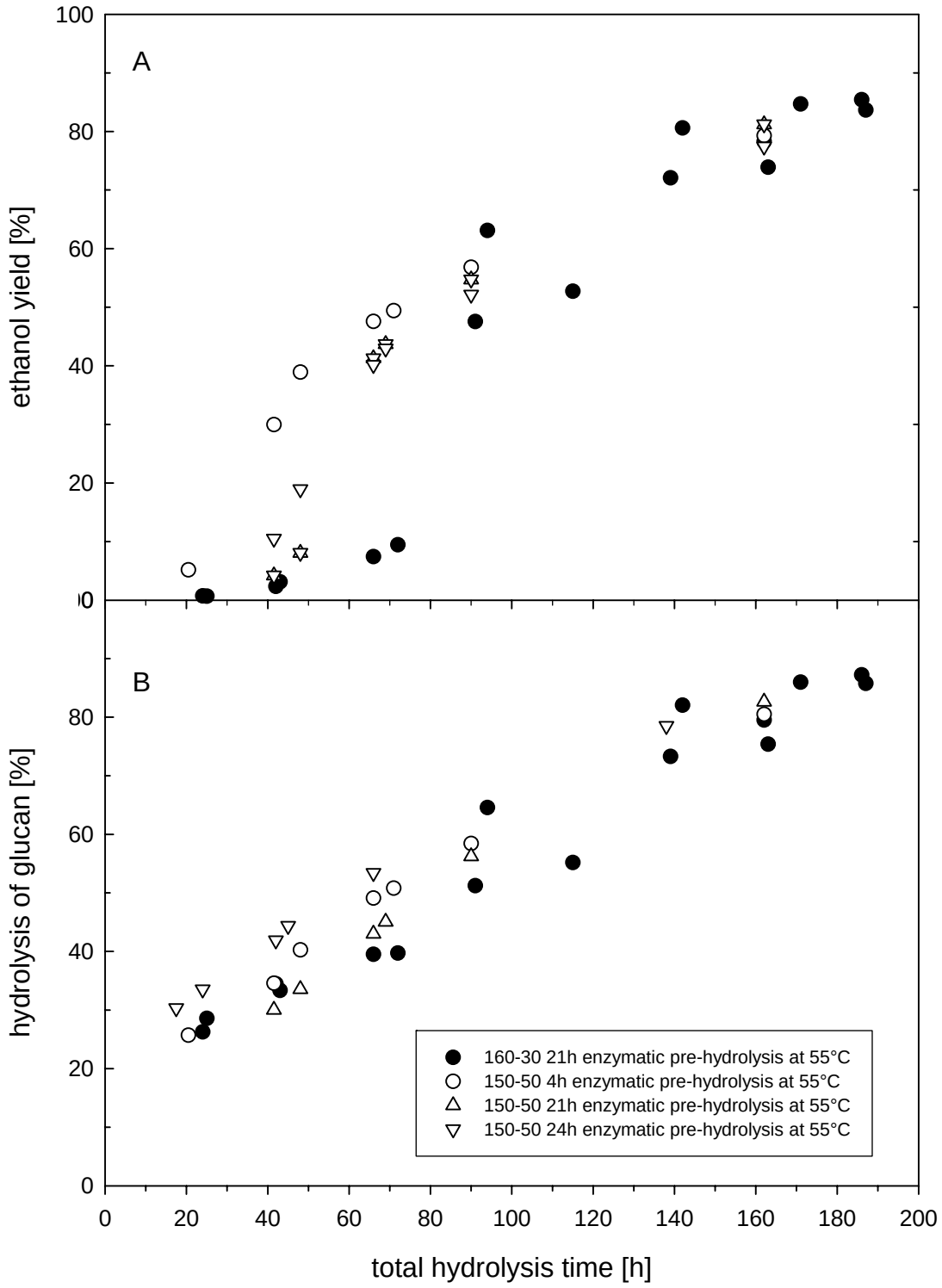


Fig. 4.1.7 The influence of the pre-treatment on the SSF process A:proportional ethanol yield during the SSF process over the total hydrolysis time, B: Proportional hydrolysis of the total glucan over the total hydrolysis time (160-30 every test series, n=4; 150-50 every test series, n=2

With regard to the ethanol yields during the experiments using the "stargen™-enzymes" on starchy material (chapter 4.4.4), here a transformation of starch to

ethanol was only calculated with a transformation rate of 90% of the theoretical because of the high dry substance that was used during the experiments.

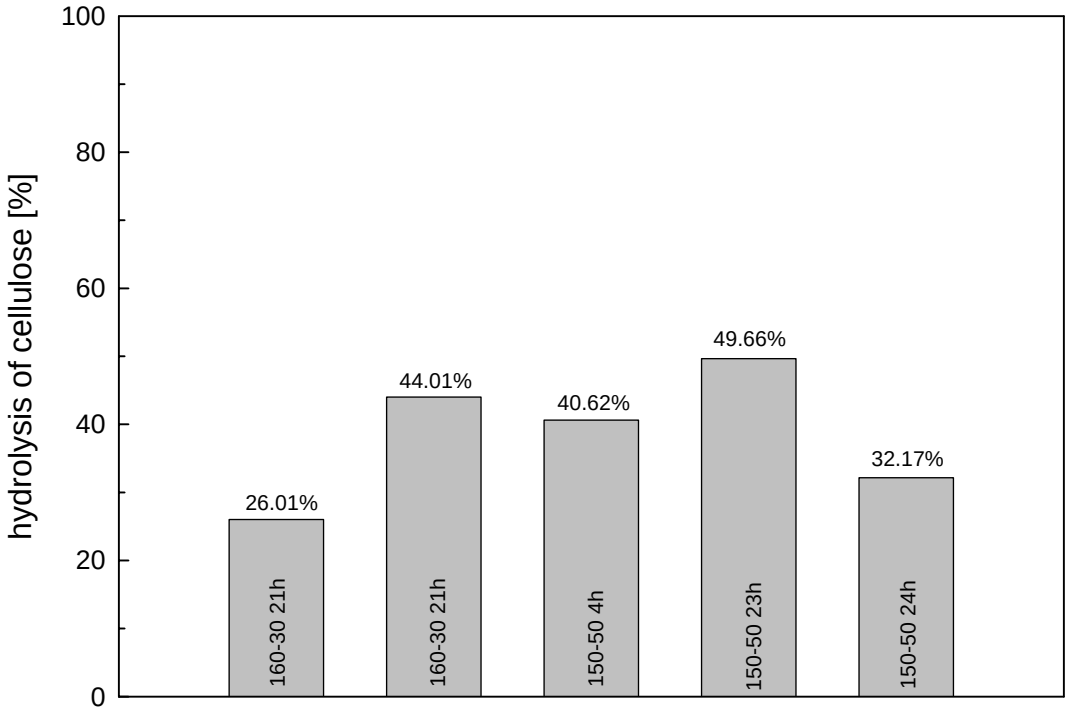


Fig. 4.1.8 The influence of the pre-treatment on the cellulose hydrolysis after ca. 162h of an SSF processes at 30°C (160-30 every test series, n=4; 150-50 every test serie, n=2)

So all glucan that was transformed to ethanol or hydrolysed to fermentable sugars, less the ethanol that was supposed to be produced from starch, is described as proportional hydrolysis of cellulose. Regarding that calculated cellulose hydrolysis (fig. 4.1.8) after 162h of total hydrolysis in the mean the pre-hydrolysis at 160°C results in a lower cellulose hydrolysis than the experiments at 150°C. But at the end of the experiment, at 187h of total hydrolysis time, the cellulose of the raw material pre-treated at 160°C results in a 71 and 77% cellulose hydrolysis respectively. This high cellulose hydrolysis yield shows that the cellulase is still active after the long process time and that high cellulose hydrolysis is possible when long process times can be realized profitable. Additionally, regarding the long process time that is needed for high glucan transformation results, the influence of the pre-hydrolysis duration is negligible. Regarding these results, the process time decides how much cellulose and glucan respectively is hydrolysed.

So the maximum process time that is economical determines how much glucan and, therefore, how much cellulose can be hydrolysed and fermented to ethanol.

4.1.6. The influence of the enzyme dosage of GC 220 on the ethanol yield during the SSF process

Enzymes, as an important factor for the hydrolysis of starch and cellulosic material, are, because of the relatively huge amount that are to be added for the cellulose hydrolysis, a very important factor for the price of the resulting ethanol. This high resulting price occurs because of the high costs for cellulases (WALPOT, 1986; EKLUND et al., 1990) because of their high dosage that is used during the process. The purpose of these analyses is to ascertain whether if it is possible to hydrolyse the pre-treated material using different cellulase dosages with the same result, in order to be able to decrease the insertion of GC 220 (Genencor, Leiden, Netherlands) for the SSF process of combined fermentation of starchy and cellulosic material. In these experiments dosages of 50µl/gDS, 100µl/gDS and 200µl/gDS respectively are used as dosage of GC 220. Before the corn silage is mashed and fermented with triticale, the dispersed corn silage is pre- treated with indirect heating at 150°C for 50min. Afterwards the pre-treated material is enzymatically pre-hydrolysed to lower the viscosity of the mash using the different GC 220 dosages at 55°C for 24h, except, for the experiments using a dosage of 200µl. This material only is pre-hydrolysed for 23h. After that pre-hydrolysis, the triticale and the "stargen™" enzymes are added and the mash is pre-hydrolysed further before the start of the SSF process. Fig. 4.1.9 shows the results for the SSF process lasting 141h (165h of total hydrolysis time). The experiments using 50µl and 100µl/g DS respectively almost result in the same ethanol yield. This can easily be explained because of the higher starch content in the mashes, and therefore, relatively low cellulose contents in the mashes. The starch content, that is about twice as concentrated as the cellulose content, is more easily transformed to ethanol than the cellulose. For the tests using 50µl GC 220/g DS in mean about 28% of the cellulose is hydrolysed at the end of the SSF process.

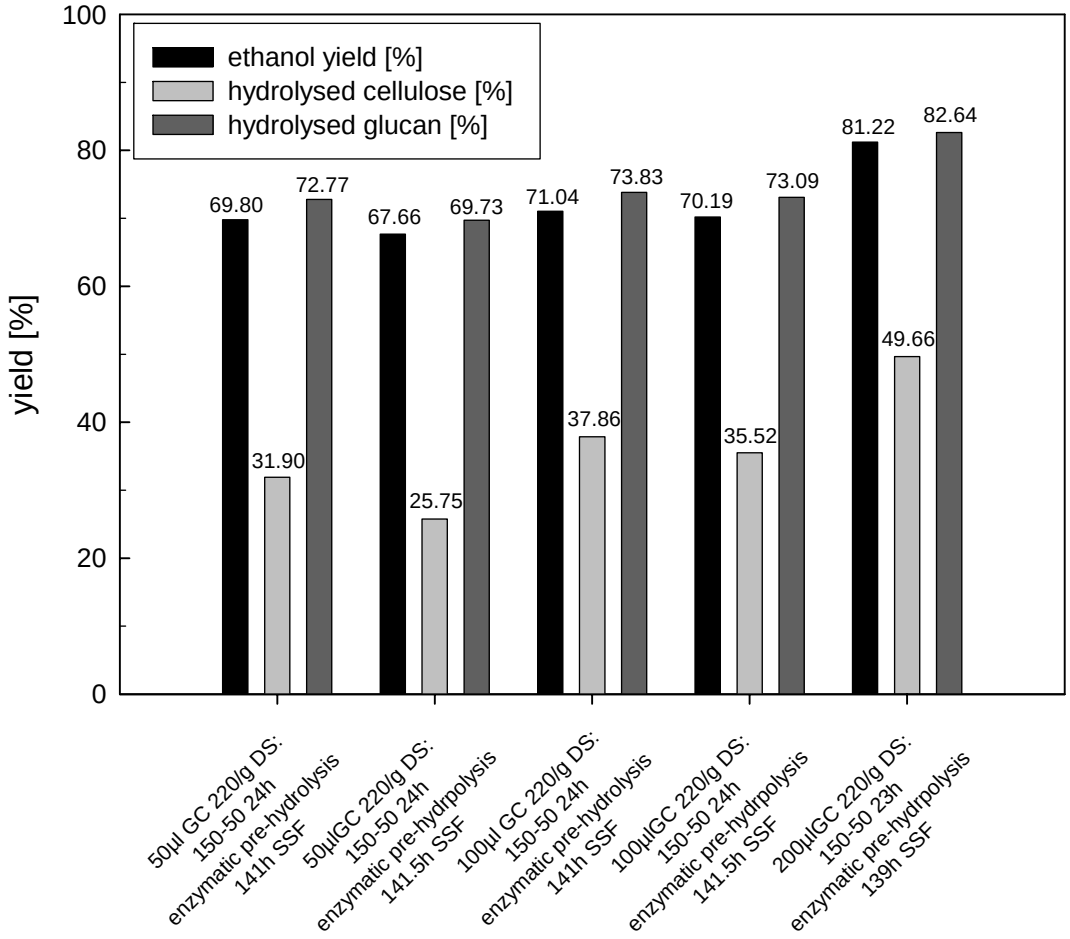


Fig. 4.1.9 The influence of the enzyme dosage on the ethanol yield after approx. 141h of SSF following an enzymatic pre-hydrolysis at 55°C for 24h (every test series, n=2)

If 100µl GC 220/g DS is used the hydrolysis slightly can be increased up to ca. 36%. In opposite to the two tests using 50µl and 100µl GC 220/g DS respectively, the tests using the highest dosage of 200µl/gDS resulted in ca. 80% of ethanol yield of the theoretical value. Also more than 80% of the total glucan could be hydrolysed and therefore, about half of the cellulose is hydrolysed. But one thing that is really remarkable is that doubling the enzyme dosage of GC 220 from 50µl GC 220/g DS to 100µl GC 220/g DS in mean results in an increase of cellulose hydrolysis of ca. 6%. If this is kept in mind, the further increase up to 200µl GC 220/g DS (4*50µl) yields a 12% higher cellulose hydrolysis yield than for 50µl GC 220/g DS. If this trend is extrapolated, ca. 560µl GC 220/g DS could theoreticalyl help to liberate all cellulose during the complete process time of ca.165h.

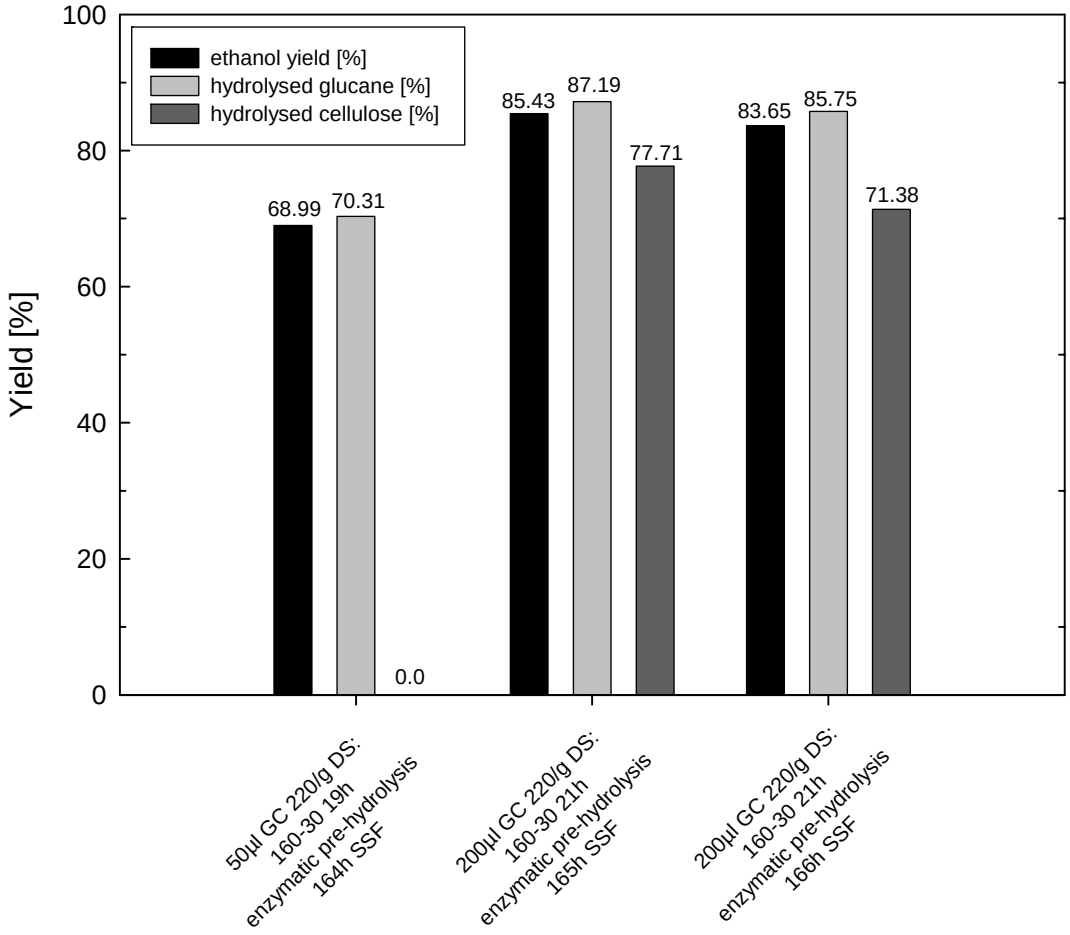


Fig. 4.1.10 The influence of the enzyme dosage on the ethanol yield after approx. 165h of SSF following an enzymatic pre-hydrolysis at 55°C for 21h (every test series n=4)

Regarding the total DS content of ca. 33% during the process in addition to the long process time, the results of the experiments using 200µl/gDS are comparable to the results that are achieved using only starchy materials (chapter 4.4.4). In addition to the starch, here ca. 50% of cellulose could be transformed to ethanol.

In addition to those experiments already mentioned, experiments are carried out with dispersed corn silage that is pre-treated at 160°C for 30min using indirect heat for thermal pre-treatment to determine the effect of the enzyme dosage on a different pre-treated material. Fig. 4.1.10 shows the results of the SSF process after 165h of fermentation (ca. 186h of total hydrolysis time). As with the experiments at 150°C for 50min, the higher enzyme dosage of 200µl/gDS resulted in a much higher ethanol yield. With the higher enzyme dosage the ethanol yield is 25% higher. In addition to the higher glucan hydrolysis rate, the calculated cellulose yield reaches more than 70% of theoretical. Also, regarding the

hydrolysis of the complete glucan (starch and cellulose), results of >85% are achieved after the long fermentation time of 164h. Using only 50µl of enzyme per g of DS no cellulose hydrolysis could be determined assuming that the starch contained in the mash is hydrolysed at a rate of 90% (based on the results of the experiments in chapter 4.4.4) during the SSF process. Using this assumption it is not possible to calculate any cellulose hydrolysis because the rate of hydrolysed complete glucan was too low. Concerning an enzymatic pre-hydrolysis for 19h and a destruction of the natural structure of the corn silage during the process (degradation of the viscosity of the mash), some cellulose has to be hydrolysed. But it was impossible to determine the correct value of the hydrolysed cellulose in the mixture of starch and cellulose contained in the mashes. With regard to the results in chapter 4.1.5 it is possible that the final hydrolysis rate and, therefore the final yield of ethanol has not been reached after the total hydrolysis time of 183h when the experiments are stopped. Longer fermentation times may result in higher ethanol yields and, therefore, in higher cellulose and glucan hydrolysis rates respectively. Consequently the cellulase dosage of GC 220 decides how long the total hydrolysis time has to last before a complete glucan hydrolysis could be reached. Or from a different point of view the maximum progress time that could be run profitably, decides the dosage of enzyme to be used and thereby the price of ethanol.

4.1.7. The influence of the enzymatic pre-hydrolysis at 55°C on the SSF process of corn silage and triticale as raw materials

In these experiments the influence of the duration of the enzymatic pre-hydrolysis at 55°C is to be examined. To realise this, dispersed corn silage is pre-treated using indirect heating at 150°C for 50min. Then 200µl of the cellulolytic enzyme GC 220 is added per gramme of DS. To reduce the viscosity of the biomass the mixture is pre-hydrolysed for different times at 55°C. To complete the combined process of corn silage and triticale as raw materials as described in 3.4.5, triticale grist and the stargen^{TM} enzymes are added and the slurry is further hydrolysed at 55°C for 2 hours. After that period, the mash is cooled to 30°C and yeast is added to start the SSF process.

All x-axes of figs 4.1.11 A, 4.1.11 B and 4.1.11 C show the total hydrolysis time of the complete process including enzymatic pre-hydrolysis with GC 220 at pH 4.2 and 55°C and SSF fermentation of starchy and cellulosic raw material at 30°C and pH of 3.5 to 4.0. In Fig. 4.1.11 A the y-axis shows the ethanol yield in % of the theoretical resulting ethanol concentration when the glucan is transformed to ethanol. With regard to the start of the SSF process, it can be seen that the ethanol production of the 4h pre-treated samples started earlier than the other longer pre-treated samples. This is due to the earlier addition of the yeast and the earlier start of the SSF process. Fig. 4.1.11 B shows the decrease of the proportional content of residual glucan in the mashes. Reciprocal proportional to the increase in ethanol yield, the content of residual glucan decreased regarding the increasing of hydrolysis time. To get an idea of how fast the ethanol is produced during the process, in fig. 4.4.11 C the average ethanol production rate [as g/(l*h)] is shown. After a lag phase of ca. 16h the yeast starts to produce ethanol. For the test using the 4 h pre-hydrolysis an ethanol production rate increased up to > 3.0 g ethanol/(l*h) is yielded after ca. 50h. At this moment ca. 40% of glucan is hydrolysed. In the following stage the fermentation speed decreases to 0.5-0.75 g ethanol/(l*h). Comparable results can be achieved for the other process using a longer pre-hydrolysis. If the time shift of ca. 20h is kept in mind, after ca. 40h the ethanol production rate accelerates. After further 36h the maximum ethanol production rate of 2.0-2.5 g ethanol/(g*h) is realized. In the following stage, as for the 4h pre-hydrolysis process, the ethanol production rate decreases to ca. 0.5g ethanol/(l*h). After ca. 80h when nearly 50% of the ethanol is yielded the pre-hydrolysis duration at 55°C seemed to have no more influence on the progress of the process. All three processes resulted in nearly the same ethanol yield regardless of the length of the enzymatic pre-hydrolysis. At this point ca. 50% of the glucan (fig. 4.1.11 B) is hydrolysed and converted to ethanol for every experiment and in each case the average ethanol production rate (fig. 4.1.11 C) also decreased. At this status of the process the ethanol concentration is ca. 50-60g/l (data not shown). So comparable to the results of LUONG (1984), the increasing ethanol concentration of the mashes could on the one hand, have had a negative correlation to the yeast growth and, therefore, a negative correlation to the average ethanol production rate.

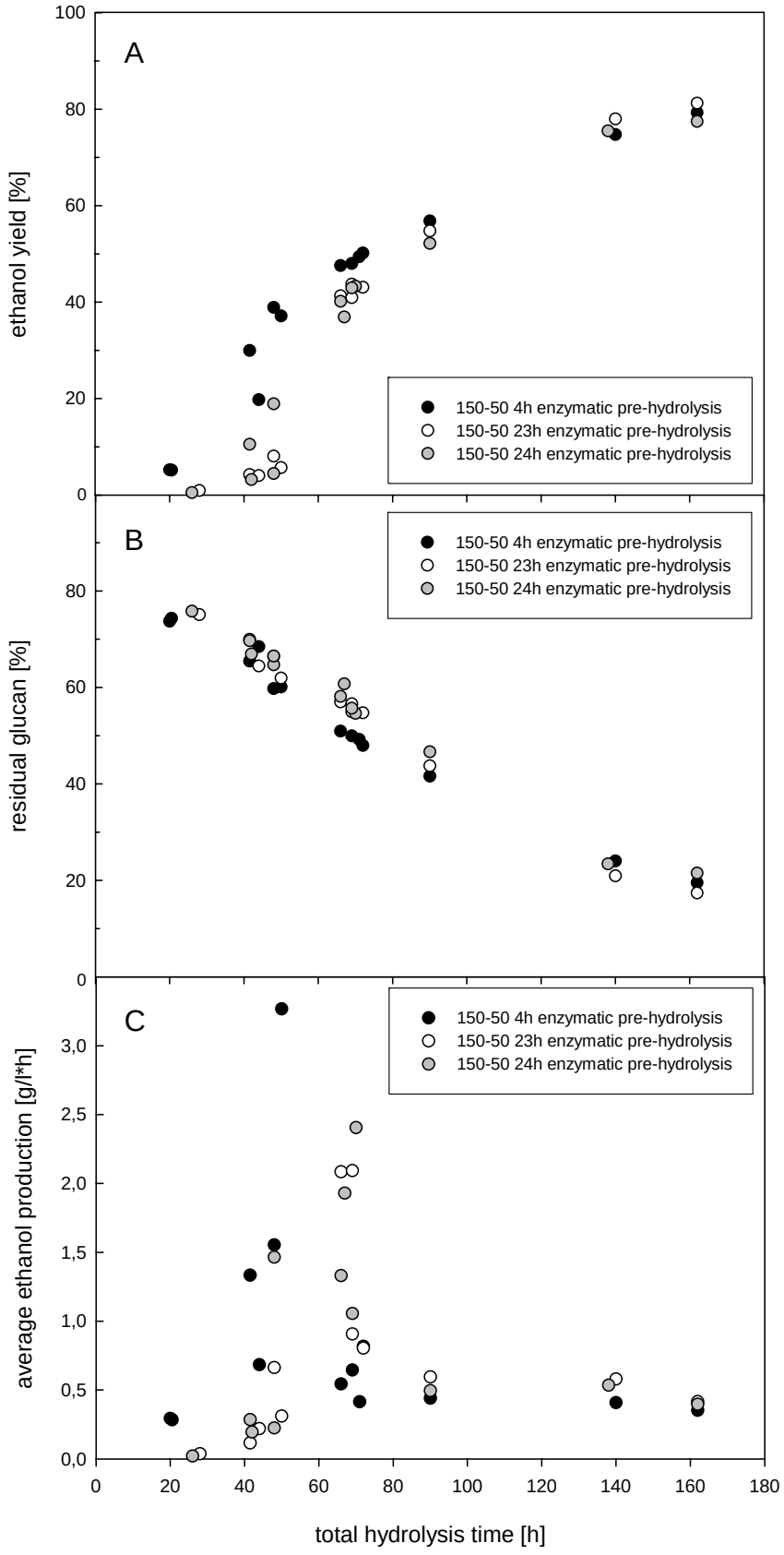


Fig. 4.1.11 The influence of the duration of the pre-hydrolysis at 55°C on A: the ethanol yield of a SSF process at 30°C; B: the hydrolysis of the residual glucan during a SSF process; C: the average ethanol production rate during a SSF process (every test series, n=2)

Additionally, the concentration of the glucan, which can easily be digested to fermentable sugars, is decreasing in the fermenting mash. Consequently the concentration of free fermentable sugars then only lay between 2 and 5g/l. This is a very low sugar concentration compared to the sugar concentrations of 30 to 60g/l of fermentable sugars, for example after 20h of the SSF process. But one fact what is very important is that although the transformation speed of sugars to ethanol is low at this phase of the SSF process (fig. 4.1.11 C), the fermentation and ethanol production process still goes on. The longer the process lasts, the more the ethanol concentration increased.

From 140h to 160h of total hydrolysis time the graphs flatten and only a little gain in ethanol yield is achieved by increasing the process time. At the end of the experiments after a total hydrolysis time of 160h, all experiments result in an ethanol yield of 76 to 82%. Considering that not only starchy material is used, this seems to be a good result. At such high dry substance concentrations that are used at the beginning of the experiments the results seem to be comparable to those that can be achieved using starchy materials only at comparable DS of 30%. Only the fermentation time is much longer. Additionally the pre-hydrolysis time of an enyzmatic pre-hydrolysis at 55°C makes no difference regarding the almost identical ethanol yields after the long process duration. As can be seen in fig. 4.1.11 C after adding the yeast and thereby starting the SSF phase of the process, the average ethanol production rate first increased with increasing hydrolysis time. The highest ethanol production rates achieved for all experiments after a hydrolysis time of 50h. Among the results, the maximum ethanol production rate is achieved for the experiment only using 4h of enzymatic pre-hydrolysis at 55°C. A further increase of the total hydrolysis time resulted in an immense decrease in the average ethanol production rate for all experiments. As already mentioned above, this can be explained because of a negative correlation of the increasing ethanol concentration with the yeast growth or because of the decreasing concentration of the more easily digestible glucan that results in a worse sugar formation and as a result, in a worse ethanol production. After a total hydrolysis time of 90h 0.5g/(l*h) of ethanol were still produced for every experiment, regardless of the enzymatic pre-hydrolysis time of the experiment. This average ethanol production rate is maintained until the end of the experiments so that ethanol is still produced at the end of the process.

A closer look at the hydrolysis of cellulose also shows that the hydrolysis of cellulose still goes on at the end of the experiments. Fig. 4.1.12 is based on the calculation of hydrolysed cellulose after a total process time of ca. 142h and 162h. To get the proportional ratio of hydrolysed cellulose, the ethanol obtained and the remaining concentration of free fermentable sugars in the mash are calculated back to their original concentration of glucan in the raw material. In the case of the ethanol yields during the experiments using the "stargen^{TM} enzymes" on starchy material (chapter 4.4.4), a transformation of starch to ethanol is calculated with a transformation rate of 90% of the theoretical because of the high dry substance content that is used during the experiments.

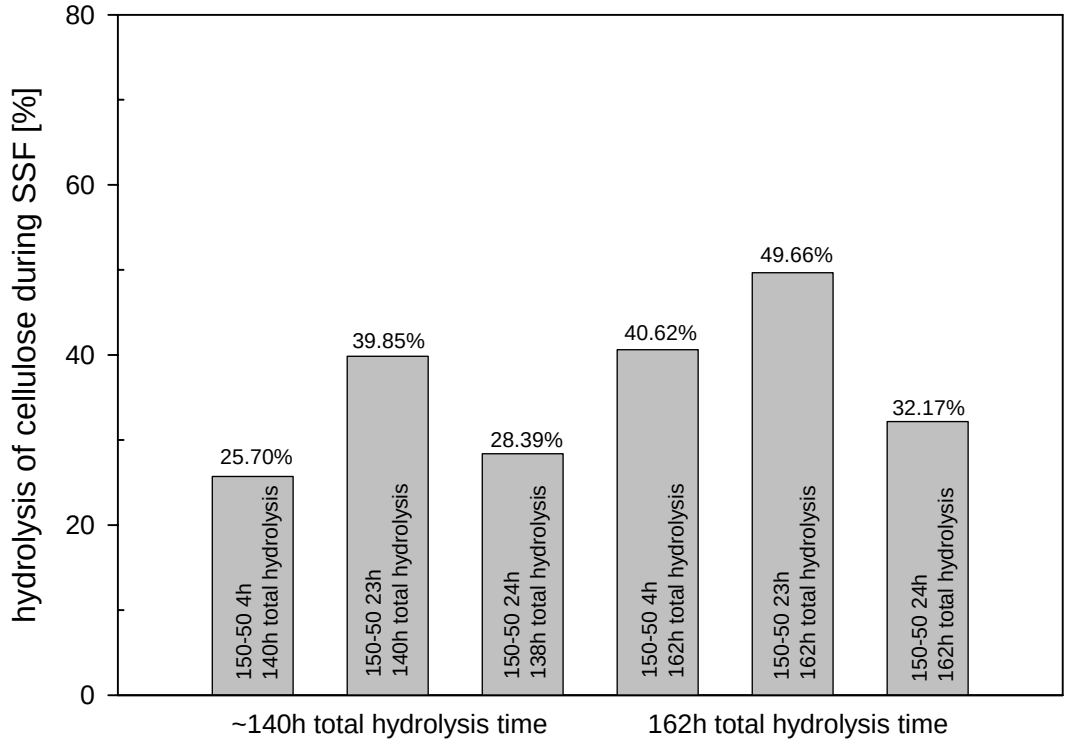


Fig. 4.1.12 Influence of the duration of the pre-hydrolysis at 55°C on the cellulose hydrolysis at the end of an SSF process at 30°C (every test series, n=2)

So all glucan that is transformed to ethanol or hydrolysed to fermentable sugars less the ethanol that is supposed to be produced from starch is described as proportional hydrolysis of cellulose. Fig. 4.1.12 shows the results of the calculation for the process duration of ca. 142 and 162h respectively. As can be seen in fig. 4.1.12, the hydrolysis of cellulose is still going on after 142h of process time. Regarding that fact, the cellulase enzyme complex still has to be active after the

142h of process duration. Refering to different studies normally the cellulase enzyme complexes tend to aggregate to the biomass and thereby are inactivated. But regarding the remaining enzyme activity of the cellulase complex after that long period of time, the combination of fermenting cellulosic biomass together with starchy biomass seemed to have the advantage that, even after such a long process time, the cellulase complexes are still active.

4.1.8. The influence of the DS on the SSF process of corn silage and triticale as raw materials

As with the SSF process using starch only to obtain ethanol concentrations that make the distillation profitable, with SSF processes using starchy and cellulosic material, the DS should be as high as possible. The reason for that goal is, that as much ethanol as could be produced in a profitable way using such an SSF process, should be produced. To examine the influence of the DS of the corn silage material during such processes, experiments are performed using dispersed corn silage that is pre-treated at 160°C for 30 min. for the enzymatic pre-hydrolysis and the SSF process.

In addition, as with the other combined experiments of starchy and cellulosic material before the start of SSF, 140g milled triticale (0.5mm sieve) is added and hydrolysed using the Stargen^{TM} enzymes. Fig. 4.1.13 and fig 4.1.14 show the progress of these experiments. As expected, the ethanol production of the tests using 9.3% DS result in the fastest and in the highest theoretical ethanol yields. After a total hydrolysis time of ca. 192h (168 h of SSF process), the maximum ethanol yield of 97% is obtained. This equals an ethanol concentration of 89g/l (fig. 4.1.14 A). Regarding the concentration of the residual glucan, the trend for this experiment shows that here the glucan is hydrolysed in the fastest way. After 20h ca. 30% of glucan is hydrolysed. A further degradation of ca. 60% can be achieved within the next 50h. In contrast to that, the test using higher DS (10.0% and 21.7% DS) after about 40h lag behind a bit. From that stage on, the glucan is hydrolysed slower than for the experiments with 9.3% DS corn silage. Even after ca. 90h these tests resulted in a glucan hydrolysis of 60%. In the same way the calculated cellulose hydrolysis resulted in the highest cellulose hydrolysis. Surprisingly the experiment using 21.7% DS produced the second best result.

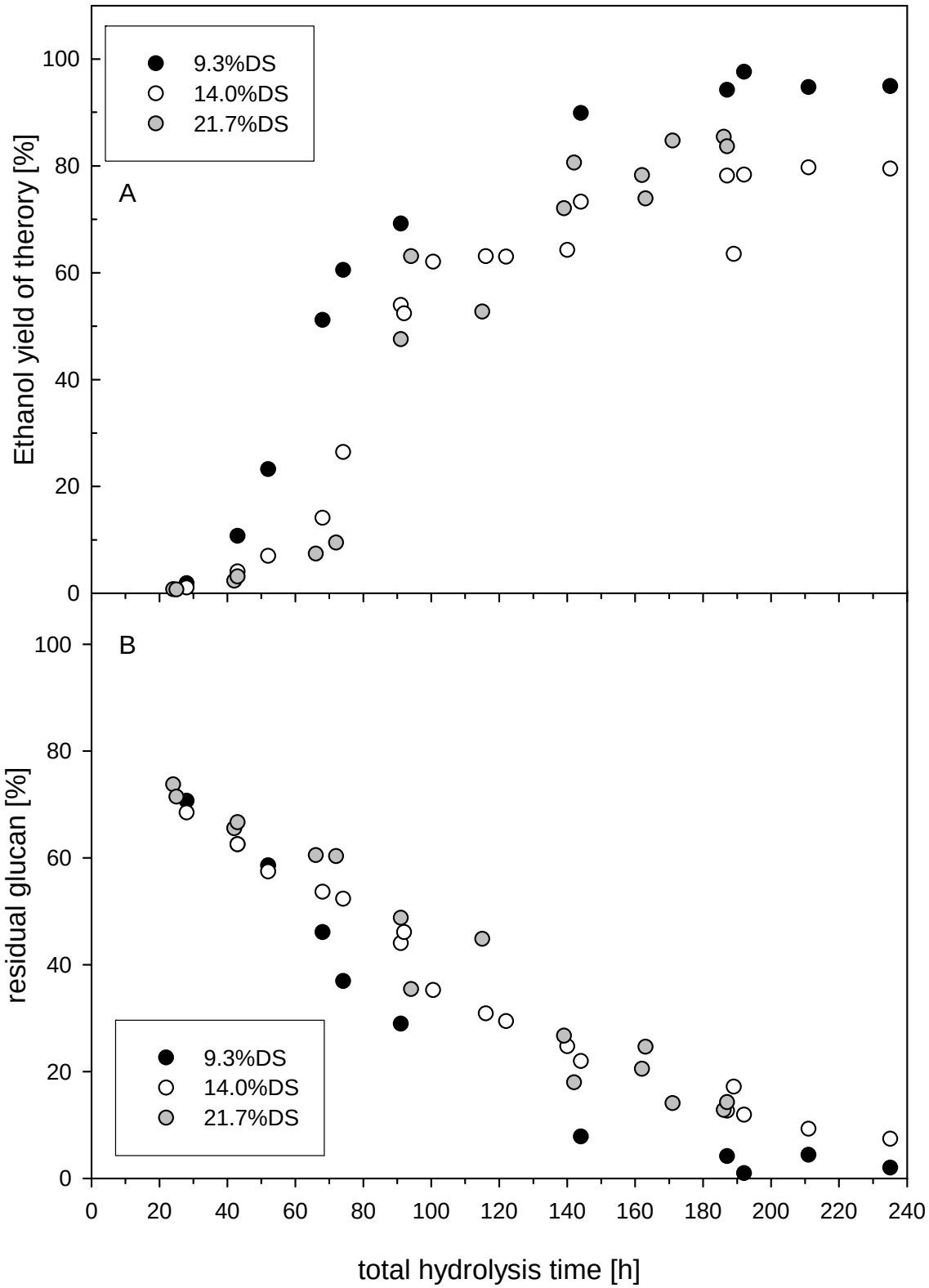


Fig. 4.1.13 The influence of the DS on the SSF process of corn silage and triticale A: The influence of DS on the proportional ethanol yield; B: The influence of DS on the hydrolysis of the residual glucan (every test series, n=2)

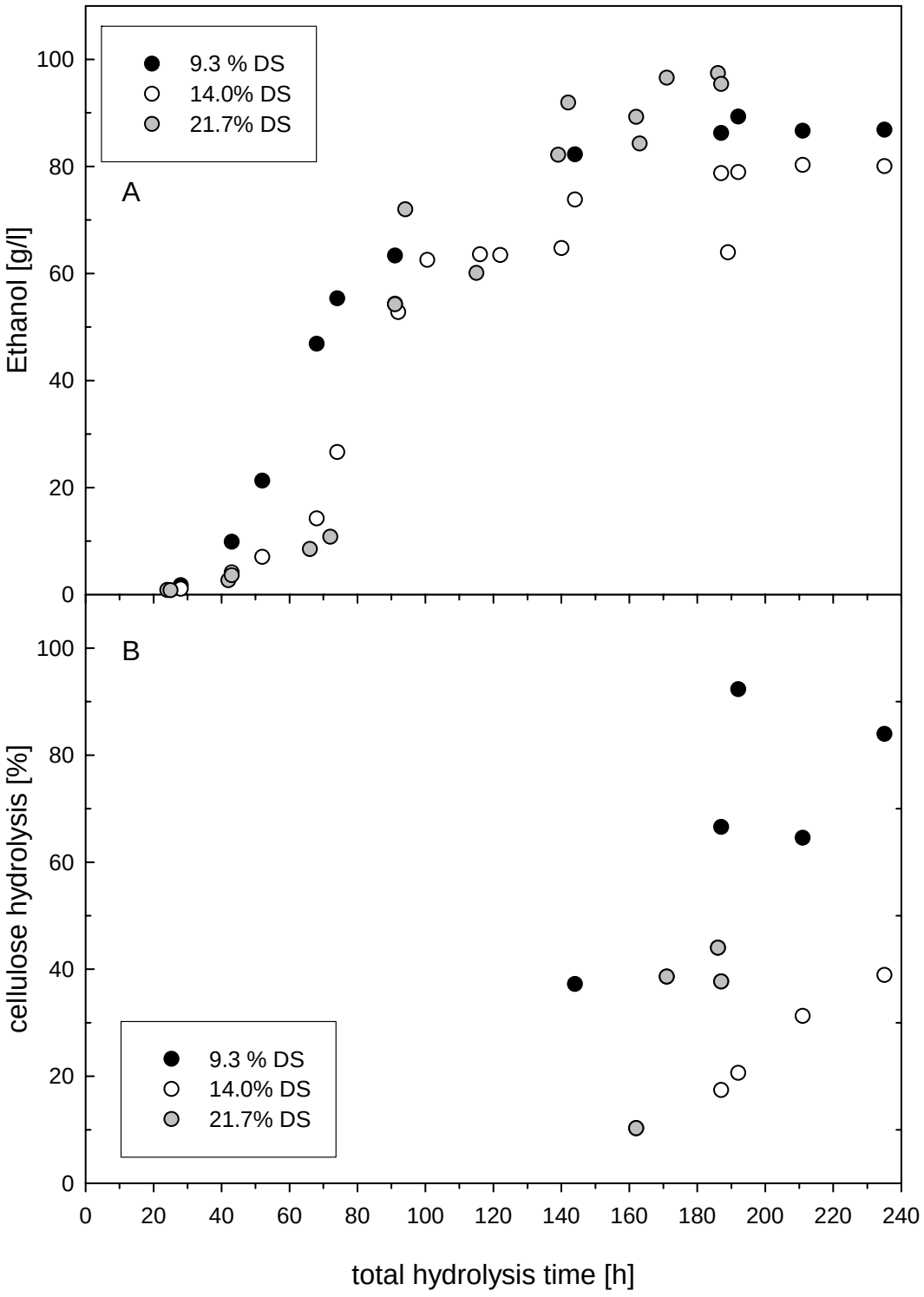


Fig. 4.1.14 The influence of the DS on the SSF process of corn silage and triticale A: The influence of the DS on the ethanol concentration; B: The influence of DS on the proportional cellulose hydrolysis (every test series, n=2)

After a slight slower initiation of the fermentation, as for the experiment with 14.0% DS of corn silage, the proportional ethanol yield catched up and in the further progress the ethanol yield exceeded the result with the 14.0% DS. At the end,

after a total hydrolysis time of ca. 171h about 84% of ethanol of the theoretical value is obtained. Compared to the result of the 9.3% DS fermentation, this is one eighth lower, but it still represents quite a good result that is comparable to those obtained in chapter 4.4 using only starch as raw material.

The experiments using 14.0% DS resulted in the lowest ethanol yield and ethanol concentration (fig. 4.1.13 A and 4.1.14 A). Even the concentration of residual glucan is comparable to that resulted using 21.7% DS (fig. 4.1.14 A). Although the total glucan content (starch and cellulose) is higher than for the experiments using 9.3% DS, the yielded ethanol concentration is lower (80g/l compared to 86g/l) at the end of fermentation (total hydrolysis time of 192h). Fig. 4.1.14 B shows that this lower ethanol concentration is due to the lowest calculated cellulose hydrolysis rate of all experiments. Regarding those results the 9.3% DS experiments result in the highest cellulose hydrolysis rate of all experiments (ca. 90%) at the end of the test. As expected, because of the obtained ethanol yield, the tests using 23.7% DS resulted in higher cellulose hydrolysis rates than the experiments using 14.0% DS. About 40% of cellulose is hydrolysed within a total hydrolysis time of 192h. At this point of time the experiment performed using 14.0% DS only result in a 20% hydrolysis of the cellulose. As can be seen in fig. 4.1.14 B the cellulose hydrolysis of these tests still continues with increasing hydrolysis time. But the yeast is not able to transform the resulting sugars to ethanol. So, although the glucan hydrolysis is still in progress, the fermentation stopped after a total hydrolysis time of 187h. But one fact that is very important to be pointed out, is the fact that all hydrolysis enzymes still seemed to work after a total hydrolysis time of 180h.

4.2. Formation of HMF and Furfural during the pre-treatment of corn silage using direct heating

After the pre-treatment of the direct heated samples, a part of the solution is collected and diluted to a tenth. This dilution is filtered and analysed in the HPLC to get an idea of how many sugars are destroyed during the pre-treatment process. The most potential (Chung and Lee, 1985; Olsson and Hahn-Hägerdal, 1996; Taherzadeh et al., 2000) and major inhibitors (Lewis, Z.L., 2006) that are produced during the sugar destruction are HMF and furfural respectively. These compounds reduce enzymatic and biological activities, break down DNA, and

inhibit protein and RNA synthesis (Sanchez and Bautista, 1988, Khan and Hadi, 1994; Moding et al., 2002). Fig. 4.2.1 shows a typical chromatogram of the hydrolysis liquor after thermal pre-treatment. After ca. 31 and 47 min HMF and furfural respectively are normally detected. But, as can be seen in the chromatogram, no peaks result for HMF and furfural. Because the calibration procedure for the HPLC analysis is made between 0.019 to 1.87g of HMF/l and 0.046 to 4.59g of furfural/l respectively maximum 0.019 g HMF/l and 0.046g furfural/l are to be contained in the pre-treated slurries. WEIL et al. (2002) mentioned that concentrations higher than 1g/l strongly inhibit fermentations and already have an effect on culture growth. So, referring to literature, the concentrations that are produced during the pre-treatments should not be a problem for a following fermentation process because they are surely lower than the inhibiting concentrations.

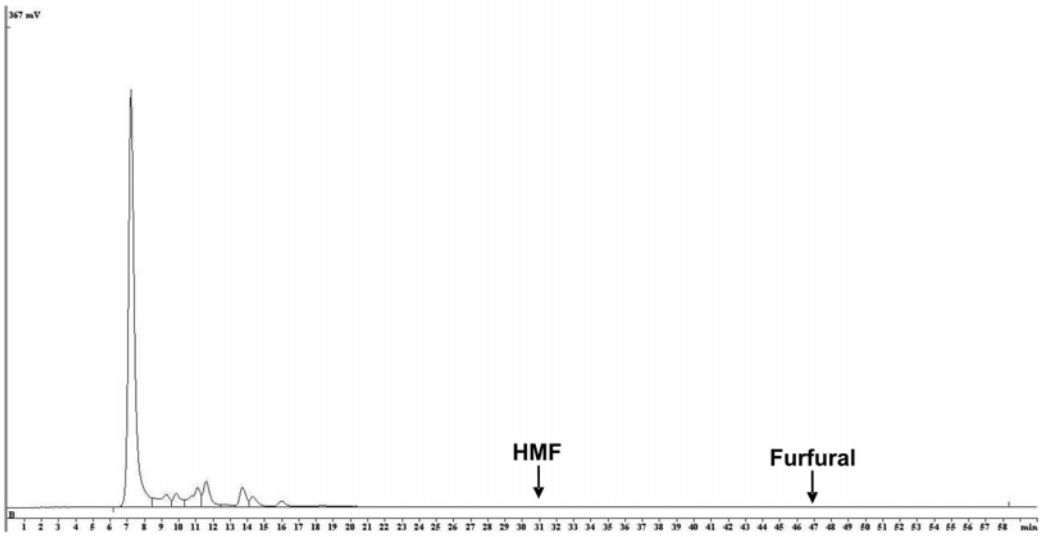


Fig. 4.2.1 Typical chromatogram of the liquor of the pre-treatment slurry

4.3. The production of bio-methane from different substrates

The stillage from the distillation process is a good substrate for the production of biogas. Moreover, by using the residues of the distillery, on the one hand it is possible to decrease residual materials while, on the other hand, by using this residue, further energy can be produced to improve the energy balance. The „Landesanstalt für Landwirtschaftliches Maschinen- und Bauwesen“ of the Universität Hohenheim friendly carried out some bio-methane experiments using different substrates. The experiments are performed using the Hohenheimer

biogas test (HBT). Table 4.3.1 gives an overview of all substrates that are tested in the HBT.

Test	Sample	dry substance [%]
1	Corn silage 2005	33
2	Corn silage 2005 dispersed and pressed	49
3	Corn silage 2006 dispersed	18
4	Stillage (Corn silage 2006 dispersed plus triticale corn)	11
5	Stillage (Corn silage 2006 dispersed plus triticale corn) plus milled triticale straw (cutting mill 0.5mm sieve)	25
6	Triticale straw dispersed	7
7	Triticale straw (cutting mill 10mm sieve)	84

Table 4.3.1 Samples for the HBT experiments

Against expectations, dispersing the corn silage (fig. 4.3.1) is not resulting in a higher methane yield (corn silage 2005/corn silage 2005 dispersed). A 8.5% lower methane yield is to be accepted. Additionally, the size reduction of the corn silage using a Becomix DH 1200 needs a lot of energy. Perhaps the pressing of the dispersed corn silage and the loss of liquor that contained some sugar resulted in the lower methane yield after the HBT. For the corn silage 2006 it is the same result. Dispersing it causes a 5.5% lower methane yield in the HBT. If dispersed corn silage from the year 2006 is mixed with triticale corn in a distillery process and fermented until no more ethanol is produced, the stillage residing after the mash distillation yielded in a bit lower methane production than the experiments using only corn silage as a substrate for the production of bio-methane. A ca. 13% lower methane yield results.

To increase the dry substance of the stillage, milled triticale straw (milled using a 0.5mm sieve) is added to the stillage. During the HBT 31.6m^3 per 100kg DS are produced. Compared to the best results for the corn silage 2005, there is a lower yield of ca. 13.2%. This is not a bad result, if the fact that ethanol from the triticale corn and the corn silage is already produced, is mentioned. Furthermore, as can be seen in fig. 4.14.1, triticale straw results in lower methane yields than corn silage and, therefore, the mixture of the stillage and the straw could not produce as much methane as if corn silage is used alone. The last two columns shown in fig. 4.14.1 are the results of the bio-methane fermentation of triticale straw. First the result of the fermentation of dispersed triticale straw is shown. By using

dispersed triticale straw for bio-methane production a higher amount of methane can be obtained compared to the experiment using the stillage of corn silage and triticale corn mixed with the straw. The result of these tests is nearly comparable to those of dispersed corn silage fermented together with corn. If the straw is not dispersed, lower methane production has to be accepted. Only ca. 27.6m^3 of methane are produced per 100kg of dry substance. This is a ca. 11% lower result compared to the fermentations using the dispersed triticale straw. So in the case of triticale straw, a size reduction results in an increase in methane production in the HBT experiments.

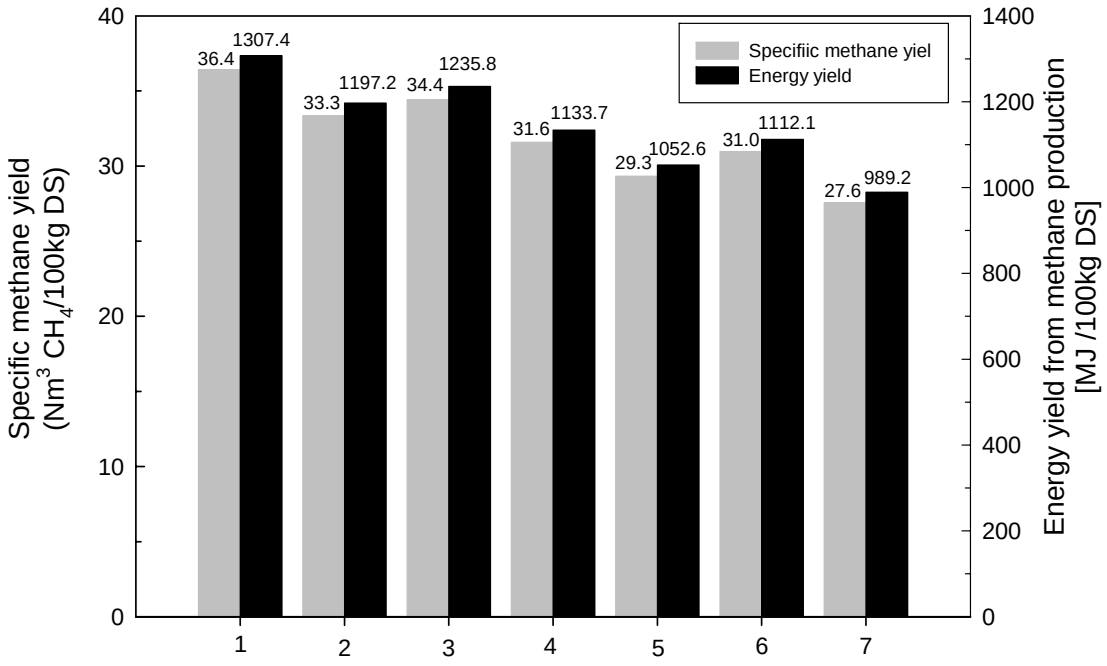


Fig. 4.3.1 Methane yield and energy yield of different substrates in the HBT experiments (only energy yield from methane production included)

4.4. Starchy materials

4.4.1. The influence of the addition of stillage and urea with or without the addition of GC 106 respectively on the fermentation of triticale

In some studies (THOMAS and INGLEDEW, 1992; JONES and INGLEDEW, 1994; KOLOTHU-MANNIL et al., 1990; THOMAS and INGLEDEW, 1992 and THOMAS et al., 1993) the fermentation could be accelerated by the addition of a nitrogen source that was accessible as free amino nitrogen for the yeast. Because

of this, results of tests using urea as a good and cheep amino resource are used to try to accelerate the fermentation for mashes with ca. 30%DS. In the tests a very high dosage of 1000mg urea/l and 500mg urea/l respectively with or without the addition of the acidic protease GC 106 (Genencor, Leiden, Netherlands) [dosage 0.232ml of a dilution of 1ml GC 106 in 25ml] are used.

Additional experiments using no further nitrogen addition and experiments using an addition of stillage are performed.

As can be seen in fig. 4.4.1 A and B, the progress of all tests is nearly the same. No significant difference between the different nitrogen contents or the other samples could be yielded. Because of the high DS during the fermentation, the results of the different tests have a very high variation. In the first 20h of fermentation, ca. 50% of the theoretical ethanol yield could be obtained for all experiments. For all fermentation experiments this is realised because of an average ethanol production rate of ca. 3.0-3.5 gEtOH/(l*h) (shown in fig. 4.4.3) during the first 24 hours. During this first period investigated, the tests using the highest urea dosage (1000mg urea/l) resulted in the highest ethanol production rate of 3.5gEtOH(l*h). Surprisingly, if no additional nitrogen is added at all, the ethanol production rate of ca. 3.25gEtOH/(l*h) is comparable to the addition of 500mg urea/l combined wih the addition of GC 106. After ca. 2 days (48h), 80 to 90% of the theoretical ethanol yield is obtained for all experiments. As for the first 24 hours of investigation, the tests using the high urea addition of 1000mg/l resulted in the highest ethaol yields. At this point of the process ca. 100-110g ethanol per litre are yielded. Because of the higher ethanol concentration the ethanol production rate of all experiments decreases to ca. 1.2-1.7gEtOH/(l*h) (shown in fig. 4.4.3). At this ethanol concentration the yeast loses significantly in viability (D'AMORE, 1992) and does not grow any more (LUONG, 1984) and therefore the fermentation speed is strongly reduced. In fig. 4.4.3 this significantly reduced fermentation speed (expressed as the average ethanol production rate over the last 24 hours) can be seen for all experiments. Because of this, during the last 24h of the fermentation tests (between 48 and 72h) the ethanol yield only increases slightly to ca. 95% of the theoretical value for all experiments. At the end of the fermentation tests the ethanol production decreases to about 0.5g/(l*h) of ethanol in all mashes. Similar effects on the ethanol production rate as shown here can be found in a patent of Genencor International (LANTERO et al., 1991).

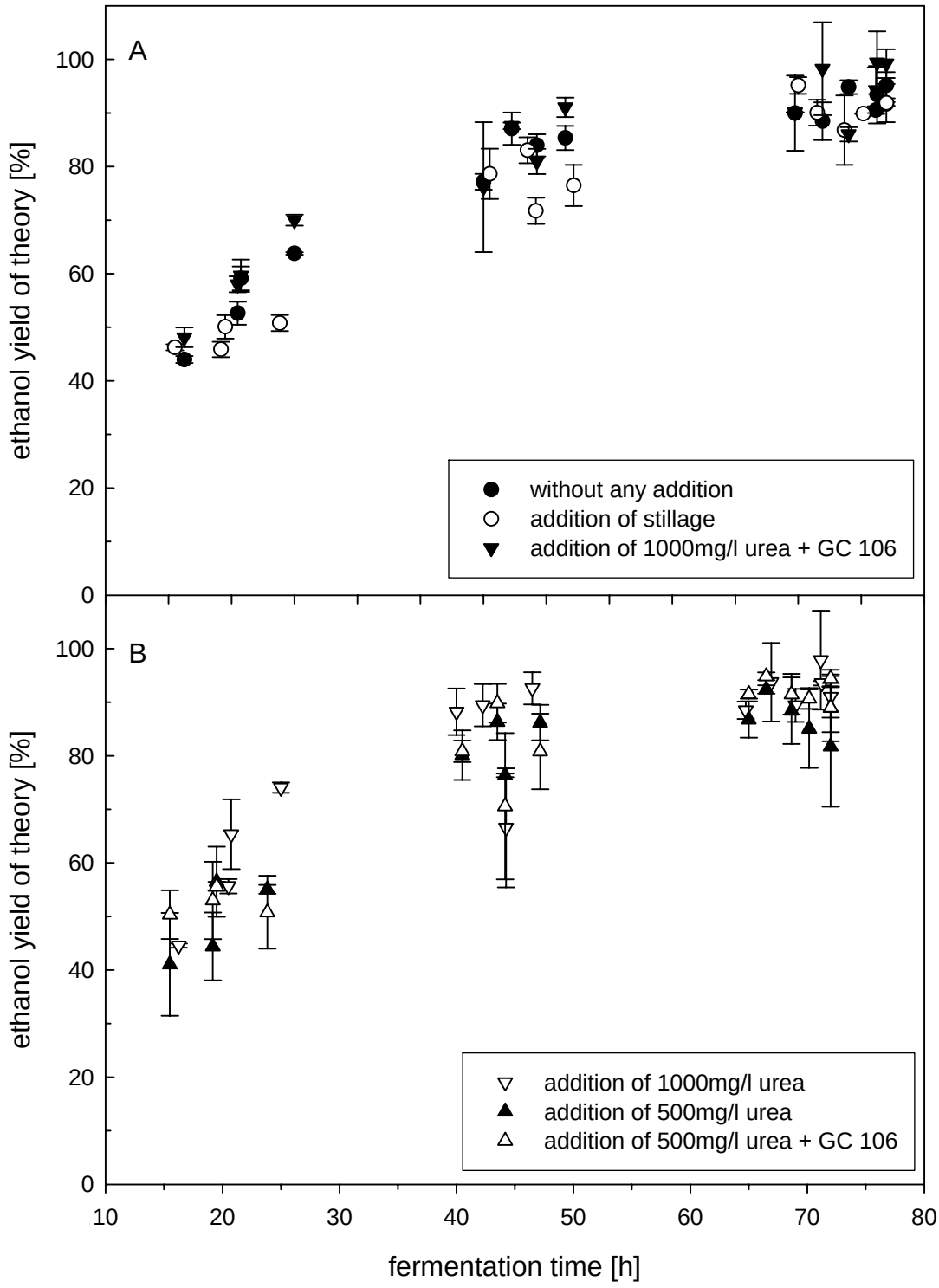


Fig. 4.4.1 The influence of the addition of A: stillage and urea or no additive at all B: urea and an acidic protease or no additive at all on the fermentation of triticale (every test series, n=2)

They also documented a decrease of ethanol production rate with increasing fermentation time. Regarding all the results that could be yielded after 3 days of fermentation, the experiments using an addition of urea of 1000mg/l during the first 48h obtained a slight higher ethanol yield than the other tests. All other test using a nitrogen addition did not result in higher ethanol yields than the tests without any addition. So even the mashes using no further addition of a nitrogen source contain enough FAN for the yeast to get as much nitrogen as it needed for it's metabolism to grow and to ferment the sugars. Only adding very high amounts of nitrogen can yield in slightly faster fermentation processes. But one thing that has to be kept in mind is that using additives means higher costs for the process. The advantage of the so called stillage recycling is that less fresh processing water has to be used for the processes. Moreover, in a distillery process heat resulting from distillation can be recycled to the process by using the stillage as processing liquid. One fact, that is very important, is the fact that the mashes that contain ca. 30% of DS could almost be nearly completely fermented after the 72h fermentation period.

Parallel to the ethanol determinations, tests of the FAN content of the mashes are performed. The results of these tests are shown in fig. 4.4.2 A and B. For all experiments the FAN content increase at the end of the fermentation. This can be explained by the declining phase of the yeasts at the end of the fermentation process. In this phase the yeasts liquefy and release their content (WANG et al., 1997). This way a lot of nitrogen is released from the cells and results as FAN in the mash. As can be seen in fig. 4.4.2, the yeast cells accumulate the FAN in the first 20 to 30h and, therefore the FAN content in the mashes decreases for all experiments. In parallel (fig 4.4.3.) the ethanol production also increases to the maximum. After ca. 40h fermentation either some of the yeast cells die and thereby releases FAN, whereby, the FAN content of the mashes increases or further FAN was formed which is not taken up by the yeast cells. At this stage of fermentation, in parallel to the increasing FAN, the ethanol production rate decreases (fig 4.4.3).

This could be caused by the inhibition of the yeast cells because of an ethanol content >100g/l (D'AMORE, 1992) or by a reduction of viable yeast cells (WANG et al., 1997).

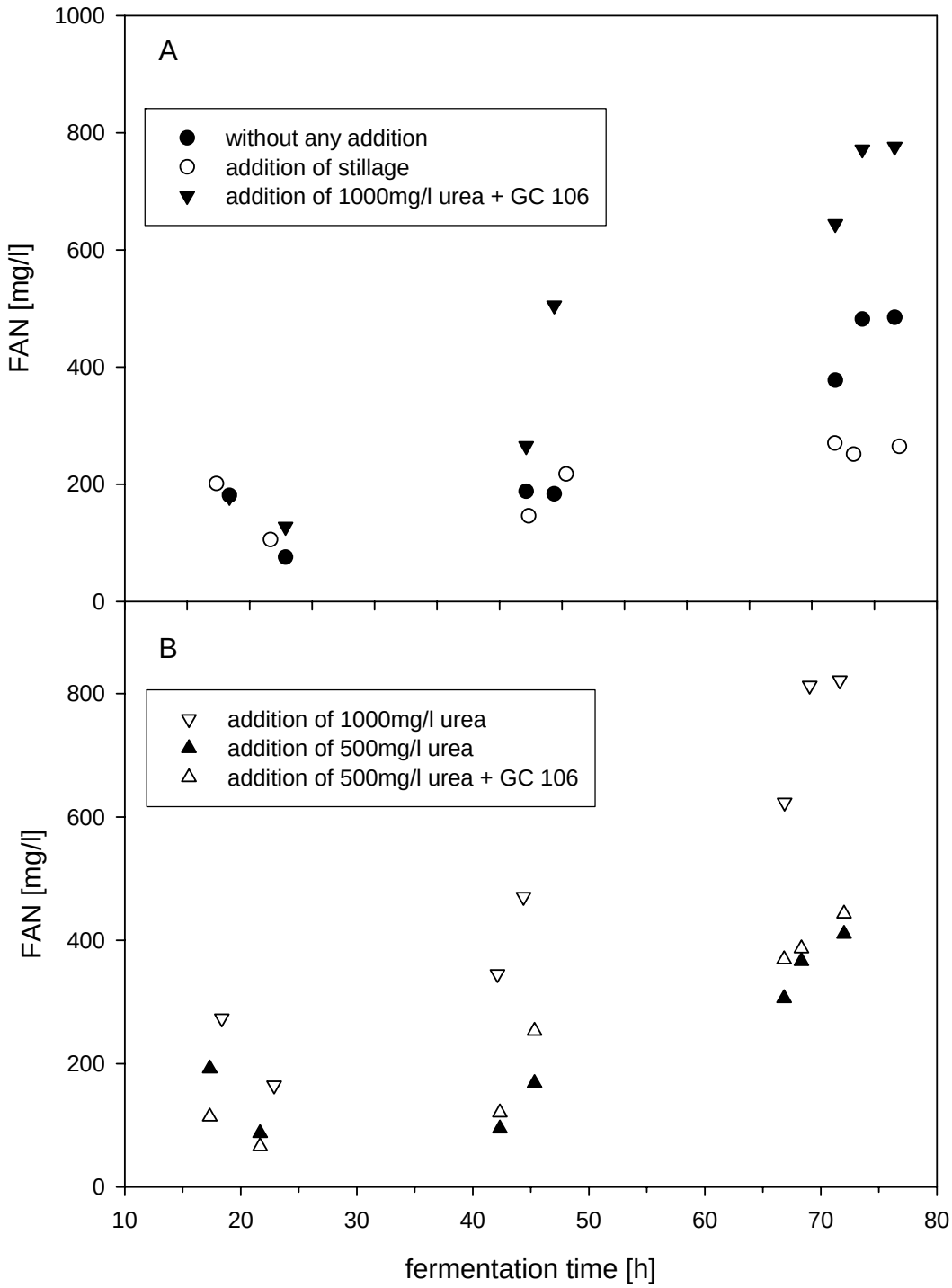


Fig. 4.4.2 The progress of the FAN during the fermentations (every test series, n=4)

The experiments using 1000mg urea/l and 1000mg urea/l and the protease GC 106 respectively have the highest FAN content (345mg/l and 265mg/l respectively) at this moment. Regarding the complete fermentation, the experiments that are performed with or without the addition of GC 106, no big difference in the progress of the FAN (fig 4.4.2) content or the ethanol production (fig 4.4.3) could be found.

So the addition of GC 106 did not lead to a higher FAN content nor to a faster or better fermentation and therefore to no improvement in the fermentation process. This may be due to the raw material, which already contains some proteases that are active during mashing and fermentation. One fact that is really interesting is the fact that the addition of stillage results in the lowest FAN concentrations at the end of the fermentation. In addition to this, the addition of 500mg urea/l surprisingly results in lower FAN concentrations at the end of fermentation than the experiments using no addition of nitrogen.

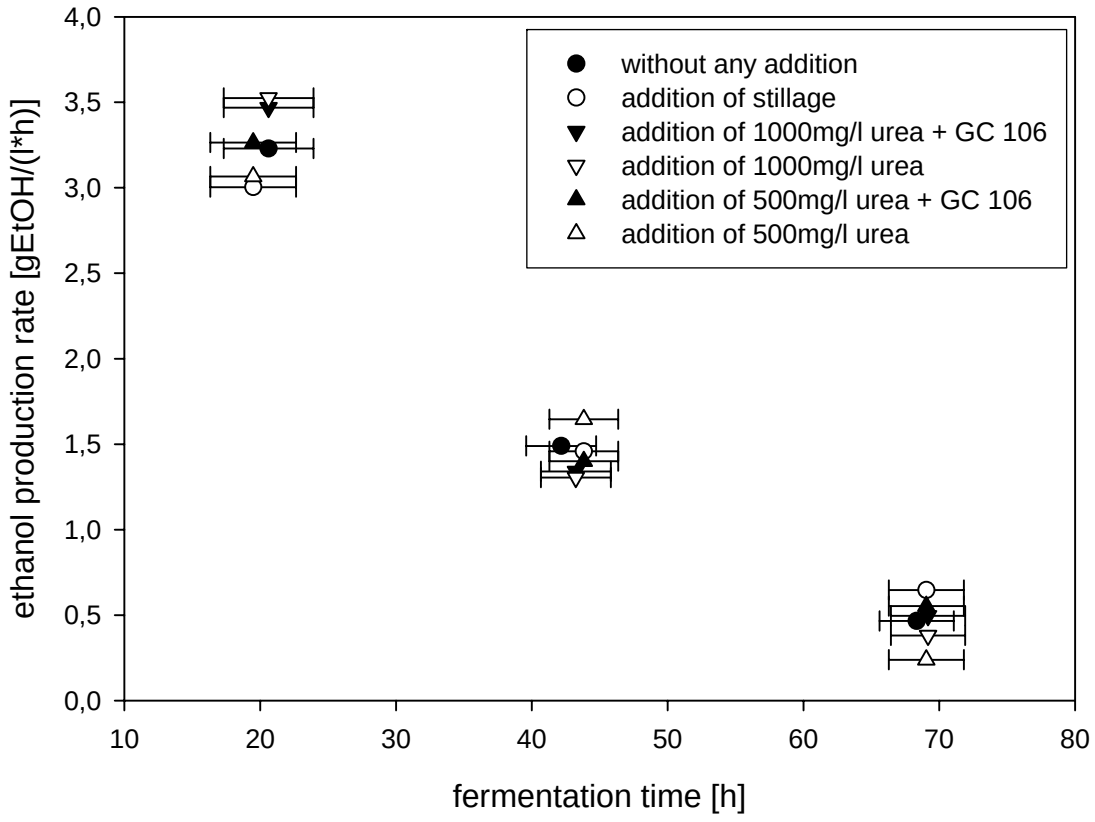


Fig. 4.4.3 The production of ethanol during the fermentation (fermentation time clustered)

4.4.2. The fermentation of wheat using different temperatures and dry substance

In these experiments the influence of the temperature on the fermentation of wheat as a starchy raw material is to be examined. Usually higher fermentation temperatures result in a faster fermentation. Under industrial conditions, for example, the ethanol is produced at temperatures ranging from 30 to 35°C (THOMAS et al., 1993) because the metabolic activity of the yeast is at a

maximum at these temperatures. Normally this fact results in the completion of the fermentation in a shorter time (JONES and INGLEDEW, 1994). Additionally the cooling requirement is less than at lower temperatures. To test if a fermentation temperature of 40°C still results in a complete fermentation after 72h, the experiments are performed with different dry substance at 40°C. To ensure that the yeast is not deactivated because of the high fermentation temperature, a special *saccharomyces cerevisiae* yeast strain called Thermosacc^{TM} is chosen, which is able to work at this conditions.

As a control, experiments using different dry substance at 30°C as the normal fermentation temperature of a distillery are also performed.

Because of the high DS, in some experiments the mashes of one series of experiments are fermented in an Inforce Laboratory Shaker to ensure a homogenous mixture. The mashes are shaken at 80 rpm. After 72h of fermentation, all resulting mashes are distilled to determine their ethanol concentrations. Figs. 4.4.4 A and B show the results of these fermentation experiments. As can be seen in fig. 4.4.4 A, fermentations at 30°C for every DS content result in the highest ethanol concentration in the mashes after 72h of fermentation. But what is very interesting is the fact that the yeast Thermosacc still seems to produce a high ethanol yield despite the high temperature of 40°C. All experiments that are performed at 40°C result, dependent on their DS, in nearly the same ethanol concentration after 72h whether they are fermented in the water bath or in the shaker. So the shaking of the mashes and, therefore, the better mixing of the mashes produces no benefit regarding the ethanol concentration at the end of the fermentation. As expected increasing the DS from 20% to 25% results in an increase in the ethanol concentration in the mash.

But increasing the DS further did not result in the expected increase in ethanol concentration for the experiments that are performed at 40°C. For dry substance of 25, 30 and 35% nearly the same ethanol concentration of ca. 11% (v/v) was yielded. Using a fermentation temperature of 30°C as expected by increasing the DS from 25% to 30% the ethanol concentration also increased to ca. 12.5% (v/v). But increasing the DS further caused a decrease in the ethanol concentration in the mash. The progress of this result is comparable to the experiments of a patent of Genencor International (LANTERO et al., 1991).

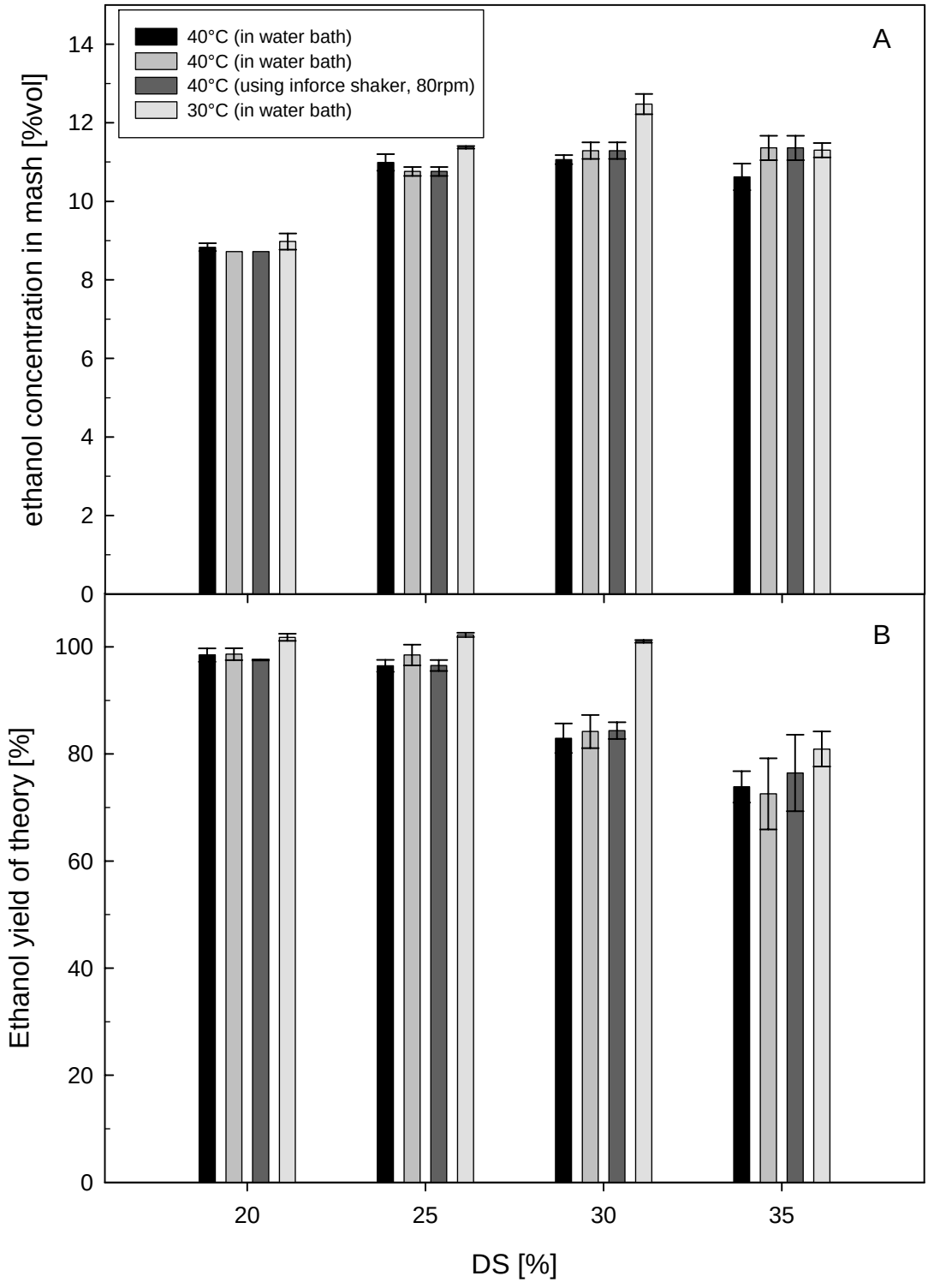


Fig. 4.4.4 The influence of DS and temperature on the fermentation of wheat (every test series, n=2)

They also had a decreasing ethanol yield during fermentation when the dry substance was increased to levels over 30% In the experiments performed using

35% DS only results in the same ethanol concentration as that yielded with the lower 25% DS. The ethanol concentration of a mash only gives the result of how much ethanol is produced by the yeast.

But these results did not explain anything about how effective the raw material is used to produce ethanol. Although the fermentation results in a huge ethanol concentration in the mash, a lot of raw material still remains unused in the mash. To get an idea how effective the processes that are performed had been and how effectively the yeast is able to transform the fermentable sugars to ethanol, fig. 4.4.4 B shows the yield of ethanol that is reached on the basis of the ethanol concentration that could be yielded in theory depending on the DS. For 20 to 30% DS using a fermentation temperature of 30°C, the process results in a nearly complete fermentation of the raw material to ethanol. When the dry substance in the mashes is increased further up to 35%, a significant loss of the ethanol yield to ca. 81% resulted.

Using 40°C as fermentation temperature for the dry substance contents of 20 and 25% the fermentations result in almost the same yield as those performed at 30°C. But increasing the DS further up to 30 and 35% respectively results in dramatic losses of ethanol yield. Only ca. 82% of the theoretical ethanol yield could be obtained at 30% DS while only 74% of the theoretical ethanol yield is obtained for the DS of 35%. This means a drastic loss regarding the usage of the raw material. About a quarter of the material is not used for the ethanol production. Regarding the results that are obtained in the experiments, fermentation temperatures at the upper limit of yeast fermentation (40°C) can only be performed effectively with a maximum DS of 25%. Mashes using a higher DS result in a drastic loss in ethanol production. This may be due to the much higher osmotic stress that affects the yeast during such fermentations in addition to the high temperature stress at 40°C. Using a lower fermentation temperature of 30°C makes it possible to perform the process using a DS up to 30%. Further increasing the DS here also results in a loss of effectiveness of the process. Although the ethanol concentration in the mashes increases when the DS is increased from 20 to 30%, the process did not result in the same effectiveness. One important fact that could be pointed out is the fact that mashes using 35% DS only theoretically produce more ethanol. In reality however, the less effective process results in an enormous waste of raw material if ethanol is the main product to be produced.

4.4.3. The fermentation of maize using different temperatures and dry substances

As with the experiments for wheat (chapter 4.4.2), the influence of the DS and of the temperature should be examined. Comparable to the tests described before, the experiments are performed at a fermentation temperature of 30 and 40°C respectively. As already before 40°C represents the upper limit for most yeasts.

The yeast product Thermosacc, which is used for the experiments, is recommended to ferment at high temperatures without any loss in activity. The other fermentation temperature (30°C) is a normal process temperature that is usually used in distilleries to produce ethanol and represents a temperature that should not lead to fermentation problems. In fig. 4.4.5 A the ethanol concentration of the fermentations after 72h can be seen. All fermentations at 30°C result in higher ethanol concentrations for all dry substance contents than the tests performed at 40°C. As expected, when the DS is increased from 20 to 25%, the ethanol concentration also increases. But increasing the DS further did not result in a further gain in the ethanol concentration. At 30°C and 30% DS results in the same ethanol concentration as for the experiments using 25% DS. And, contrary to the expectations, the highest DS concentration, results in a decrease of ethanol concentration compared with the experiments using 25 and 30% DS respectively. In the case of the fermentation temperature of 40°C, nearly the same ethanol concentration of 8.5% (v/v) is obtained for all experiments. As with the experiments performed at 30°C, increasing the DS from 20 to 25 and 30% DS respectively results in an increase of the ethanol concentration in the mashes.

But unlike these experiments, fermentation at 40°C did not result in a comparable increase of ethanol concentration. If the proportional ethanol yield based on the theoretical ethanol yield is regarded, it could be seen that the increase in ethanol concentration that could be yielded during fermentation at 30°C was not high enough. Fig. 4.4.5 C shows that, for all experiments, the proportional ethanol yield decreases when the DS is increased. This is the case whether the fermentation is performed at 30 or 40°C. In addition to the decrease of proportional ethanol yield resulting from increasing the DS, the experiments that were performed at 40°C always resulted in dramatically lower ethanol yields.

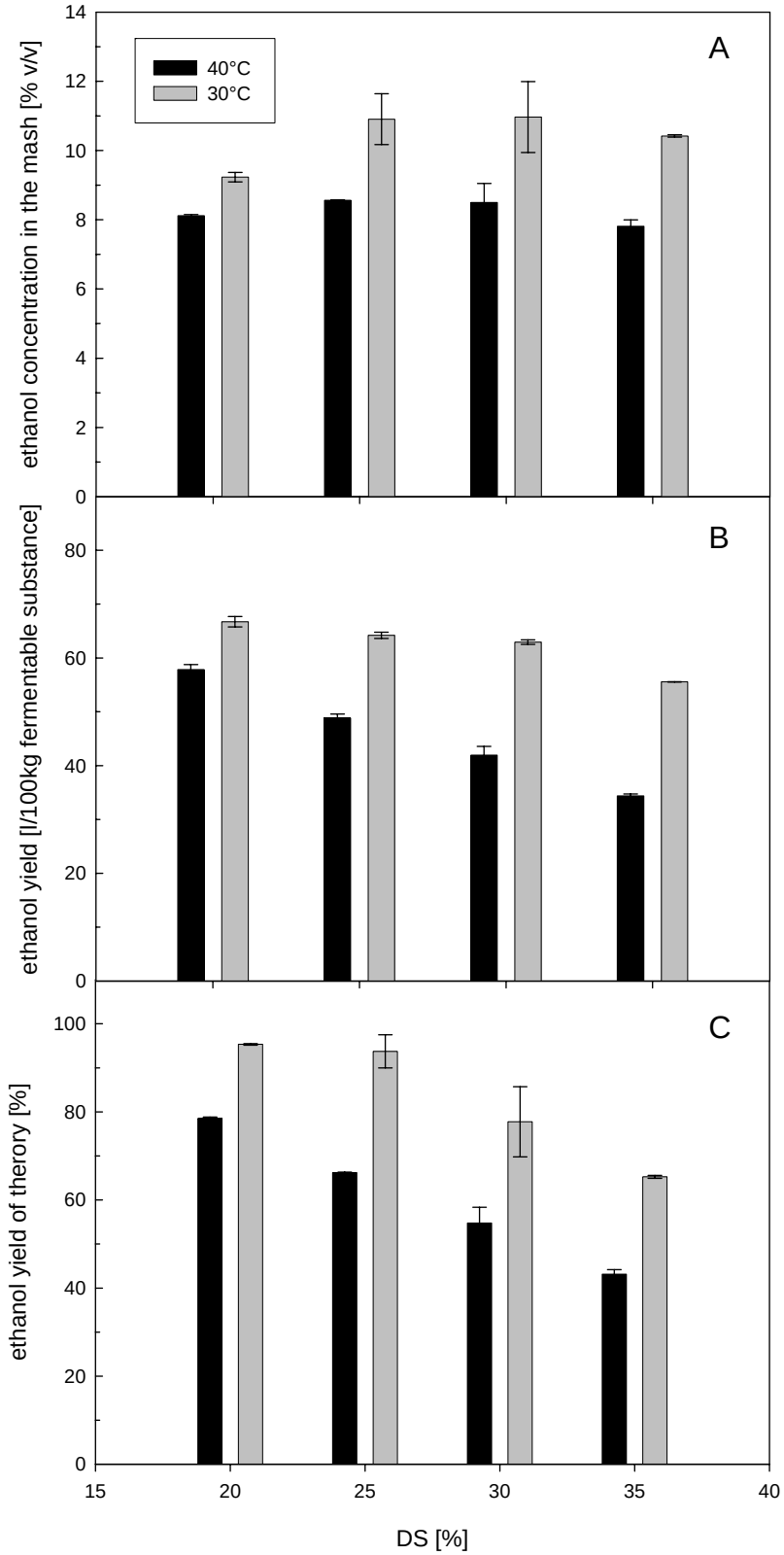


Fig. 4.4.5 The influence of DS and temperature on the fermentation of maize (every test series, n=2)

Only the ethanol yields of the tests using 20% DS at 40°C are comparable to the tests that are carried out at 30°C using 30% DS. Similarly the ethanol yield for 25% DS is comparable to the results that are obtained at 30°C using 35% DS. Increasing the DS further up to 30 and 35% DS respectively only yields in about 50% proportional ethanol concentration. Consequently, only 50% of the material could be used at dry substances of 30 or 35%. With the ethanol yield that could be obtained per 100kg of fermentable substance (FS), which represents a data that is comparable to the starch content, the same trend that can be seen in fig. 4.4.5 C for the proportional ethanol yield, can be seen in fig 4.4.5 B. Increasing the DS results in a decrease of the ethanol yield per 100kg of FS. In the same way, as shown before, the experiments using a fermentation temperature of 40°C all result in lower ethanol yields. The higher the DS, the greater the gap between the ethanol yields at 30°C and 40°C. Only the experiments using 20, 25 and 30% DS respectively and 30°C resulted in suitable ethanol yields. The experiments that are performed using 35% DS at 30°C deviated too much so that too much material remains unused in the mashes. And the experiments that are performed at 40°C showed that maize mashes could not be fermented at 40°C because the loss of ethanol was too high and, therefore, the effectiveness of the process is too bad for such a process to be used in a distillery. The best results that could be achieved using maize as a raw material were the experiments that are performed at 30°C using 20 and 25% DS respectively. These experiments theoretically resulted in ca. 8.5 to 11.0 % (v/v) of ethanol and, therefore, represented mashes that could easily be fermented within 3 days.

4.4.4. The influence of the DS on the ethanol yield during an SSF process using the Stargen™ enzymes

In these experiments the influence of the dry substance on the ethanol yield during an SSF process was examined. But in difference to the tests discussed before here a novel enzyme system, which is known as Stargen™ enzymes, is used for starch digestion. As already discussed in chapter 2.2.2, using the Stargen™ enzymes means working at temperatures below the point of gelatinization of the starch and consequently digesting granular starch. With triticale, which is used for the experiments, the optimum temperature that could be used, is ca. 55°C.

Fortunately, this is the same temperature that is to be used for hydrolysing cellulosic corn silage using the cellulase complex GC 220 (Genencor, Leiden, Netherlands).

If the idea to process starchy and cellulosic materials for ethanol production in one mash, for a complete distillery process, would be kept in mind, these experiments were performed at the ideal temperature range of cellulose hydrolysis (55°C) and granular starch liquefaction respectively.

Fig. 4.4.6 A shows the ethanol yields that result during the fermentation process when using different dry substances. The experiments using 25% DS yield in the fastest fermentation over the complete process time, although the ethanol production rate is the lowest at the beginning of the process (fig 4.4.7 B), and produce in the highest ethanol yield (96%) of all experiments, which is a much better result compared to other studies of WANG et al (2005) and WANG et al (2007). The higher the DS is, the slower the ethanol is produced, although the ethanol concentration and therefore the relative ethanol yield increases during the fermentation time. But as can be seen in fig. 4.4.7 B, after an increase in ethanol production rate in the first 20 hours up to ca 2.0 g/(l*h), what represents quite goods results compared to other studies using the Stargen^{TM} enzymes of WANG et al (2005) and WANG et al (2007), for all tests, in the following progress all fermentations slow down concerning the ethanol production rate. But what is quite interesting, the increase in fermentation speed of the experiments at 37% DS lag behind in comparison to the others (fig. 4.4.7 B). The reason for this can be seen in fig. 4.4.7 A. During the first stage of fermentation at 37% DS the yeast produces more glycerine what is caused by the higher osmotic stress for the yeast cells.

In the case of the highest DS examined (37% DS), the maximum ethanol yield obtained is reached already after ca. 83h of fermentation time and only results in 73% of the theoretical value (fig. 4.4.6 A). This result stands for a loss of about a quarter of the ethanol that could be obtained. Parallel to the increasing ethanol yields, the remaining glucan concentration decreases (fig. 4.4.6 B) during fermentation. Regarding the glucan that results after the fermentation process at 37% DS (fig. 4.4.6 B), 22.5% of the glucan remained unused in the mash. This corresponds to 20.8 g/l of fermentable sugars (maltose, glucose and fructose) that still remain in the mash at the end of the test.

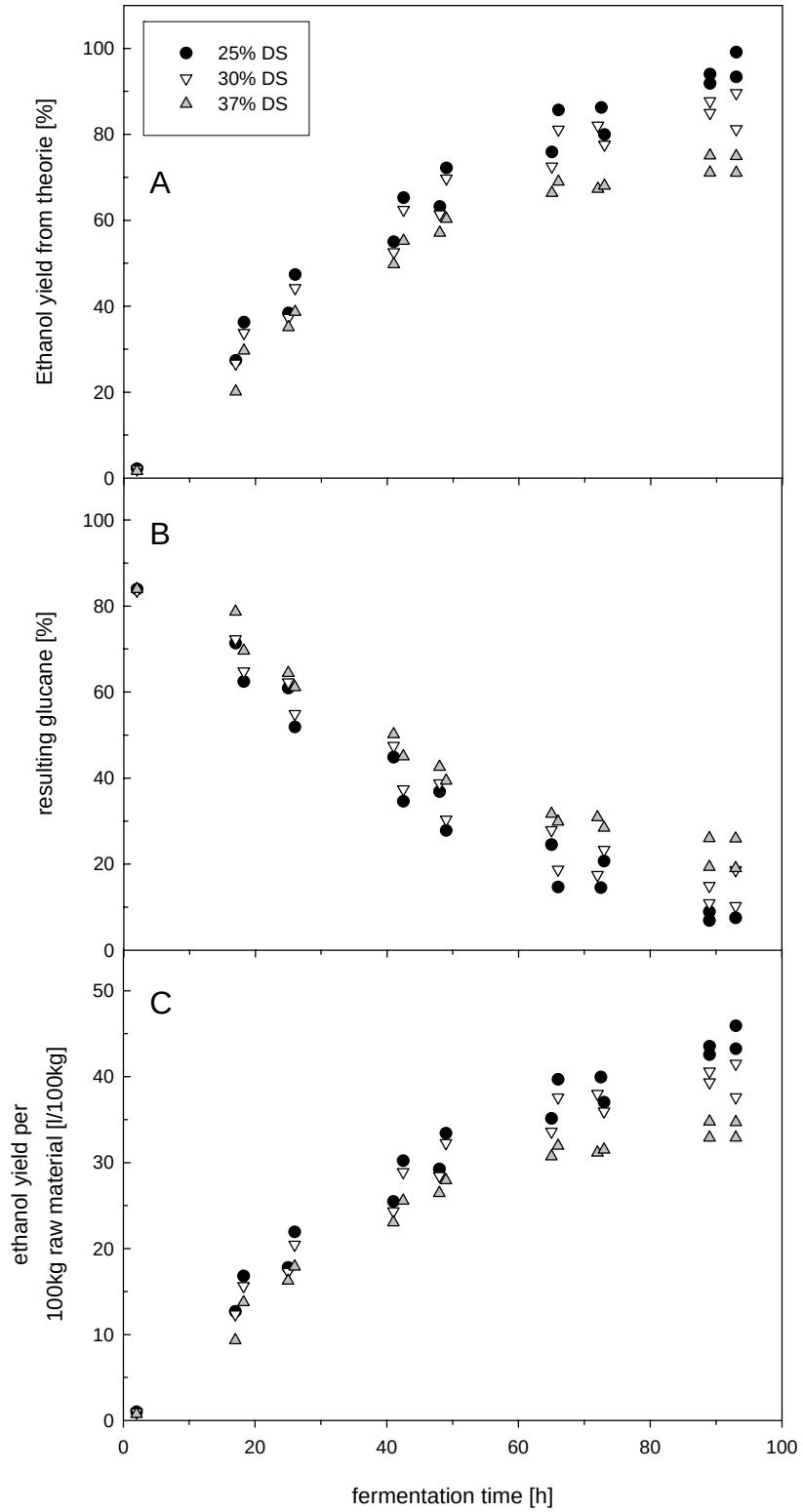


Fig. 4.4.6 The influence of the DS on the SSF process of triticale using the „stargen“ enzymes. A:resulting ethanol yield during the process; B: residual glucan during the process; C: ethanol yield per 100kg of triticale during the process (every test series, n=2)

This is not a really effective process because nearly a quarter of the glucan and ca. 10g/l of potential ethanol remained in the mash after the fermentation process. Unlike these experiments, the fermentation accomplished using 25% DS only resulted in a glucan residue of 3.7% that corresponds to ca 5g/l of fermentable sugars. This is much more effective because nearly all the glucan could be used for the production of ethanol. Additionally, the transformation of the sugars to ethanol did not stop at 83h so that ethanol is also produced after more than 83h. With a 30% DS content 14.5% of glucan (6,7g/l of fermentable sugars) still reside in the mash after the fermentation for 93h.

Consequently only 85% of the theoretical ethanol could be yielded at the end of the experiments. Considering the fact that in maximum a mash using a DS of 30% resulted in 125g/l of ethanol, thought should be given to the advantages of trying to produce higher ethanol concentrations when the raw material could not be used completely. Using even higher DS, like in the experiments using 37% DS, produced even worse results. Fig. 4.4.6 C shows the ethanol yield for all experiments calculated on the basis of 100kg of triticale. As can be seen a maximum DS of 30% resulted in an ethanol yield of 40l or more per 100kg of raw material. With 37% DS only 34l of ethanol could be yielded per 100kg of triticale. If such low raw material usage combined with high raw material prices would occur in a distillery the process would not be profitable.

But in contrast to that, regarding the concentrations of glycerin for all experiments (shown in fig 4.4.7 A), one positive result can be pointed out. In contrast to normal ethanol production processes that result in typical glycerine concentrations of ca 1.2-1.5% (RUSSEL, 2003) at the end of the fermentation, here for all tests only 0.6% of glycerine can be determined (fig. 4.4.7 A) at the end of the process. One reason for this could be the low osmotic stress for yeast over the complete SSF process.

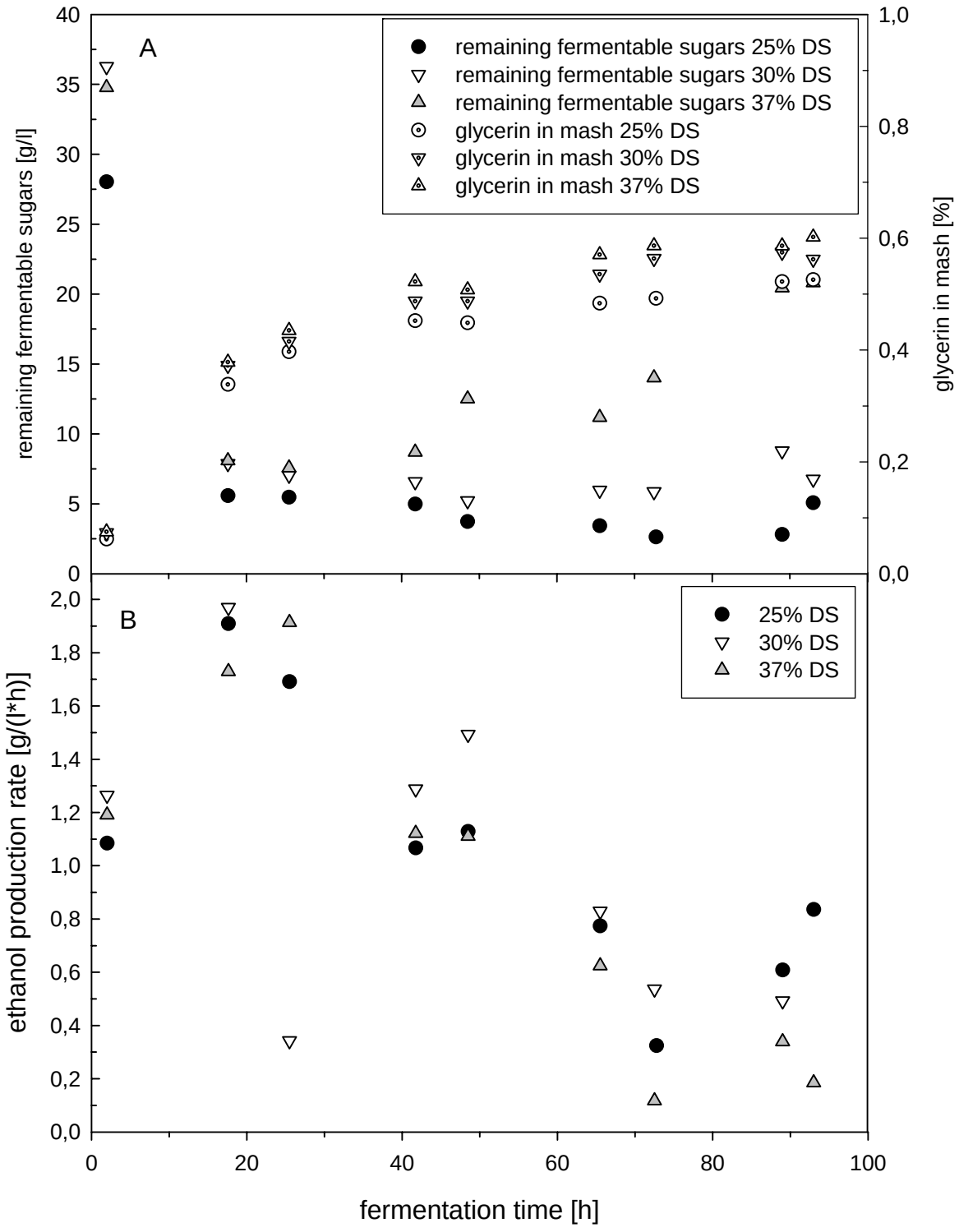


Fig. 4.4.7 The influence of the DS on the SSF process of triticale using the stargen™ enzymes. A: remaining fermentable sugars and glycerine concentration in the mash during the process B: ethanol production rate during the process (every test series, n=2)

If all the results are regarded, and therefore the ethanol yields and the effectiveness of the processes, it could be said that dry substances that are higher

than 30% result in drastic losses of ethanol and process effectiveness. A maximum DS of 30% promises processes that could yield in ca. 40l of ethanol per 100kg of raw material or more. So trying to enhance distillery processes by increasing the DS results in more process problems during the pumping and distilling and may cause a drastic waste in the utilisation of the raw material as well as a higher utility demand. Using ca. 25% DS and thereby trying to produce mashes of ca. 100g of ethanol per litre results in complete and safe processes that can be finished within 3-4 days.

5. Ecobalances

5.1. The balance of potential distillery processes

To find out which kind of biomass usage is the best regarding the emission balance and the energy gain, different scenarios of biomass usage are described in the following. These scenarios are compared with each other regarding their energy yield and their ecobalance. Basis for all scenarios is an agricultural model farm which uses ca. 1000 hectares of farmland. To ensure that a sustainable process is used for agriculture, triticale and energy maize are cultivated in crop rotation. To supply biomass to the farm throughout the year two thirds of the farmland is cultivated with triticale. On average an amount of ca. 7 tons dry substance of corn and triticale straw per hectare are calculated as yield. In addition to the triticale, energy corn is cultivated on the remaining third of farmland. In the case of energy corn the potential yield is estimated at 20 tons of dry substance per hectare. In addition to the agritechnology, the model farm owns a distillery and a biogas plant with block heat and power plant (BHKW). The stillage resulting from the distillery process and the material of the harvest not used in the distillery process are used as substrate for the biogas production. For the ecobalance two different methods of energy supply for the distillery are intended. One possibility is to supply the energy requirement of the distillery with self-produced energy from the BHKW. There some of the biogas produced by the biogas plant could be used to produce electricity and heat for the distillery process. Using this method of energy supply means that the complete process is fed autarkic. The other possibility is to supply the energy requirement of the distillery using fossil energy. In both cases the resulting biogas and ethanol that is not needed in any process step will be brought to market.

5.2. Balance sheet classification

In all scenarios the model farm uses the same agricultural techniques for energy maize and triticale production. An existing process module of the balancing software GaBi 4.0 (PE-Europe) was used to balance the triticale production. This

process module contains a very complex agricultural model which contains all environmental influences, losses of fertiliser etc. Because no such module existed in the software a new one had to be modelled for corn silage. Fig. 5.2.1 shows the flow chart of the process and all sub-processes that were included in the modelling. First of all the maize has to be sown using a single corn sowing. With this sub-process the consumption of diesel and the environmental emissions for the agritechnology are balanced. The seed that is needed for the process has to be bought and is not included in the balance. Also the cultivation of the seed and all the emissions that are produced during this process are not included in the ecobalance. Furthermore, the manuring of the biogas residues and mineral fertilizers as well as pest management are kept in mind. The energetically combustion and emissions are also included in the module. To ensure that enough fertilizer is calculated for the complete agricultural process, mineral fertilizers were taken in consideration. To accommodate the fact, that a part of the fertilizers is lost because of eluviation or other environmental influences, a loss of 10% is assumed. For the production of the fertilizers already existing modules of GaBi 4.0 were used. Consequently, all important processes should be included in the modelling of these processes. Furthermore, the harvest of the maize, the storage of the maize in the silo and the transport of the silage to the distillery also have to be balanced. As with for the other process steps, the emissions and the energy consumption of these processes also have to be modelled.

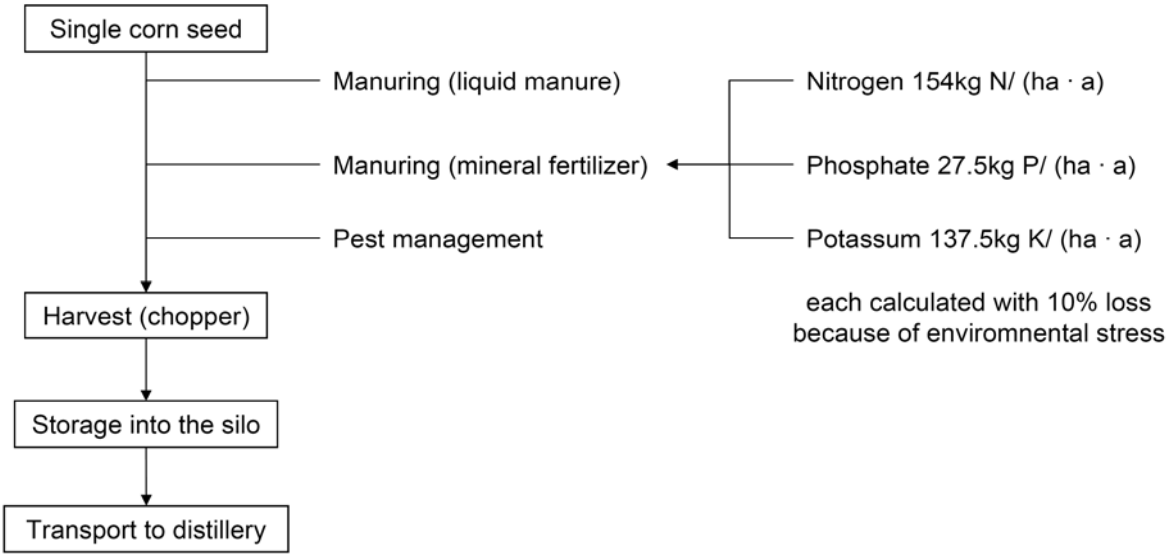


Fig. 5.2.1 Balance sheet classification of the production of maize silage

5.3. Distillery process using triticale corn to produce ethanol and usage of resulting stillage, triticale straw and maize sillage in the biogas plant

The first scenario that is to be described is a process which is similar to the classical distillery process of a sustainable ethanol production. Here only the starch-containing triticale corn is used during the distillery process. The stillage resulting from this process is used as substrate for the biogas production. In addition to the stillage, all other agricultural products of the crop rotation that could be harvested are also used for biogas production. Fig. 5.3.1 represents the flow chart of the complete production process. First the harvested triticale corn is processed to separate any impurities and dry it for storage. Afterwards the corn is ground using a hammer mill and hydrolysed using the already mentioned Stargen^{TM}-enzymes. To make sure that the fermentation can nearly be completed, the mashes are calculated using a ca. 25% dry substance and are to be fermented for 93 hours before they can be distilled. In the ecobalance the energy requirement for the distillation is calculated combined for the distillation and dehydration of the ethanol. Here data from the study by SENN (2002) is used. As already mentioned above, the resulting stillage of the mash distillation process is mixed together with the recovered triticale straw and the silage of the energy maize production and transferred into the biogas plant to produce bio-methane. The value of the digested residue of the biogas plant as a fertilizer is not calculated in the balance, because no proper data was available. Accepting a recommendation of PE-Europe, the energy of the digested residue was disregarded. Additionally, a complete fertilization of the cultivation already was modelled with mineral fertilizers. Because no data was available for the supply of the energy requirement of the agritechnology using self-produced energy, such as bio-methane or bio-ethanol, fossil fuels were modelled for these process steps. As already mentioned above, the energy requirement of the distillery can be supplied in two different ways. On the one hand, the energy (electricity and heat) can be provided with the aid of the BHKW (electrical degree of efficiency 30%, thermal degree of efficiency 40%) using a part of the bio-methane. In the following this method is called “autarkic distillery 1”. On the other hand, all energy (electricity and heat) is produced using fossil fuels. In the following this method is called “fossil distillery 1”.

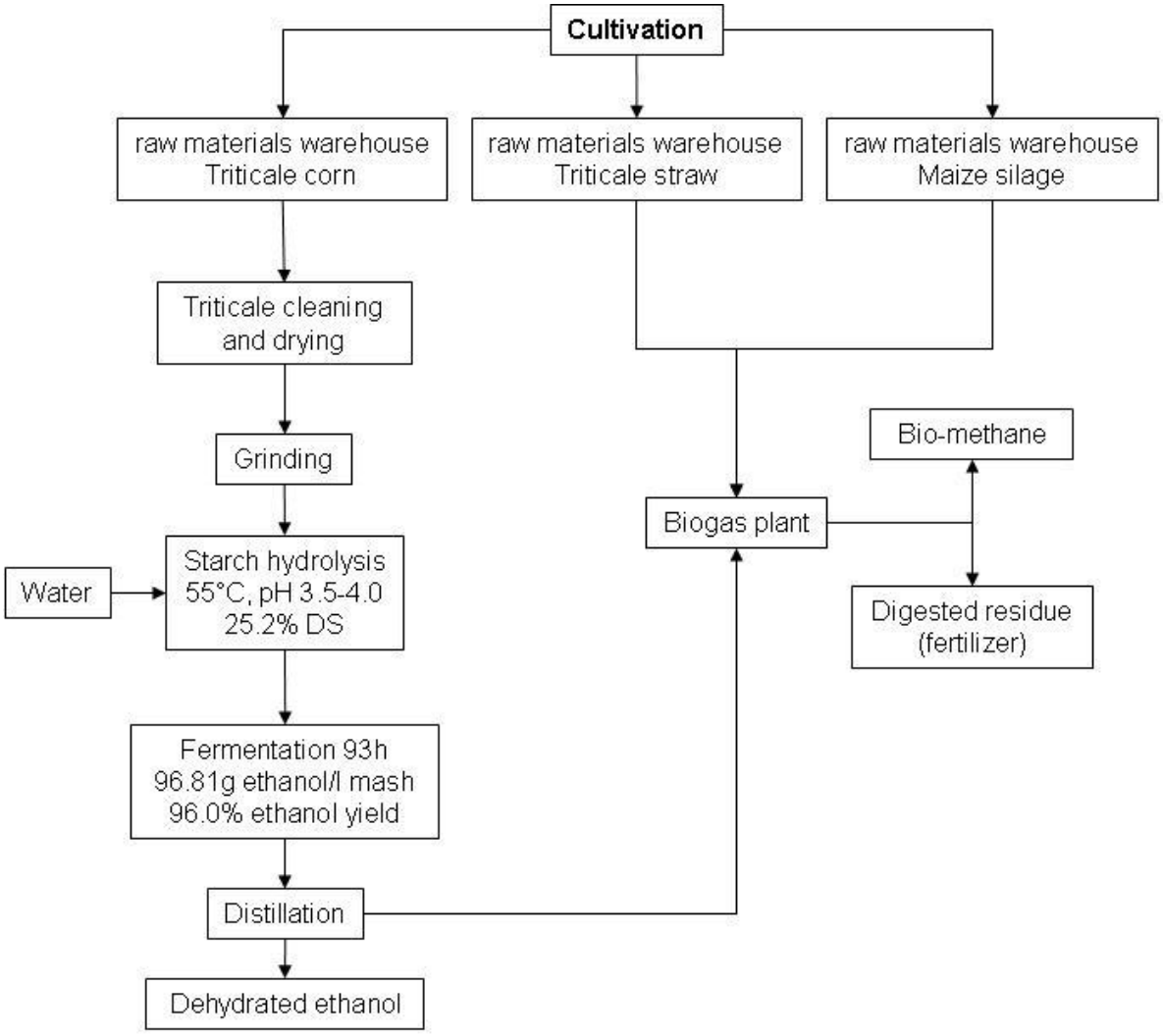


Fig. 5.3.1 Distillery process only using triticale corn to produce ethanol

Table 5.3.1 shows the potential of the two different scenarios. The balance shown represents the energy balance per hectare. As could be expected with the usage of fossil fuels for the complete process (fossil distillery 1) a greater energy demand results than for the process when heat and electricity for the distillery is produced in the BHKW (autarkic distillery 1). This is because producing electricity from fossil fuels based on the “deutscher Strom-Mix” needs much more energy compared to the process where the electricity on the model farm is produced from bio-methane using the BHKW.

	[MJ per ha · a]		
	Autarkic distillery 1	Fossil distillery 1	Difference (fossil distillery – autarkic distillery)
Input			
Energy demand cultivation Triticale	15 919.1	15 919.1	0
Energy demand cultivation Maize	2 453.3	2 453.3	0
Energy demand distillery	13 694.3	18 683.0	4 988.7
Sum energy input	**32 066.7**	**37 055.4**	**4 988.7**
Output			
Surplus electricity	7 049.7	0	-7 049.7
Ethanol	50 554.1	50 554.1	0
Methane	116 289.2	145 923.2	29 634.0
heat and electricity from BHKW for distillery	13 694.3	0	-13 694.3
Sum energy output	**187 587,3**	**196 477.3**	**8 890,0**
Energy gain (energy output – energy input)	**155 520.5**	**159 421.8**	**3 901.3**
Energy gain : Energy input	**4.85**	**4.30**	
Energy output : Energy input	**5.85**	**5.30**	

Table 5.3.1 Energy balance for the complete process only using triticale corn in the distillery

If fossil energy is used for supplying the distillery process (fossil distillery 1), the energy gain of the process that could be obtained is 3901.3 MJ/ha higher than with the other method of operation (autarkic distillery 1). This is because the energy output of this process (fossil distillery 1) is ca. 8890 MJ/ha higher, which is caused because of the higher amount of bio-methane that results at the end of the process. However it is necessary to remember that no usage of the resulting bio-methane or ethanol is included in the balance. Losses resulting from the transformation of this energy during their use, as it already was calculated for a part of the energy products for the other method of operation (autarkic distillery 1), here is not included, because only the caloric value of the end products ethanol and bio-methane are taken into consideration. Considering that fact, the bigger energy gain compared to that obtained using an autarkic fed distillery process is relatively low. By way of contrast, if the ratio between the energy input (sum energy input) and the energy gain is regarded, the autarkic distillery process results in a ratio of 4.85 to 1 (energy gain : energy input). On the other hand, the fossil fed distillery process (fossil distillery 1) only results in a ratio of 4.30 to 1 (energy gain : energy input). Thus, the autarkic distillery process is the more

effective distillery process compared to the fossil distillery process (fossil distillery 1). As a result of the lower temperatures that are needed using the Stargen™ enzymes, extraordinary high 4.30 MJ of energy could be yielded from 1MJ of fossil energy. But if the energy requirement of the distillery is supplied autarkic (autarkic distillery 1), whereby a part of the energy products is lost at the end of the process, it is possible to produce up to 4.85 MJ of energy from 1 MJ of fossil energy. This way ca. 0.55 MJ more energy could be yielded. This is an increased relative gain of ca. 12.8% compared to the operating method with fossil energy only.

In addition to the energy balance, an ecobalance of the process was made. The ecobalances (table 5.3.2) of both methods of operation show that the autarkic-fed distillery process (autarkic distillery 1) not only has a better eutrophication potential, but also a better acidification and better greenhouse gas potential than the fossil fed distillery process (fossil distillery 1).

	per hectare · a		
	Fossil distillery	**Autarkic distillery**	**Difference** (fossil distillery - autarkic distillery)
Emissions into fresh water	**CML2001, eutrophication (EP); kg phosphate-equipollent**		
Analysis values of the emissions into fresh water			
Biochemical oxygen demand (BOD)	0.0002	0.0001	<0.0001
Chemical oxygen demand (COD)	0.0526	0.0235	0.0291
Complete dissolved organic bound carbon	<0.0001	<0.0001	<0.0001
Complete organic bound carbon (TOC)	0.0041	0.0034	0.0006
Inorganic emissions in fresh water			
Ammonia	0.0070	0.0070	<0.0001
Ammonium / Ammonia	0.1216	0.1211	0.0005
Nitrate	13.4018	13.4015	0.0003
Phosphate	0.4794	0.4792	0.0002
Nitrogen	<0.0001	<0.0001	<0.0001
Organically bound nitrogen	0.8326	0.8326	<0.0001
Organic emissions into fresh water			
Hydrocarbon into fresh water	<0.0001	<0.0001	<0.0001
Hydrocarbon (unspecific)	0.0001	0.0001	<0.0001
Methanol	0.0084	0.0084	<0.0001
Oils (unspezific)	0.0007	0.0005	<0.0003
Xylene (Isomers; Dimethylbenzol)	0.0004	0.0004	<0.0001
Emissions into air			
Anorganic emissions into air			
Ammonia	2.6576	2.6568	0.0008
Ammonium	<0.0001	<0.0001	<0.0001
Ammonium nitrate	<0.0001	<0.0001	<0.0001
Nitrogen oxides	1.2355	1.0877	<0.1478
Nitrate monoxide	0.1731	0.1731	<0.0001
Summation	**18.97**	**18.80**	**0.18**

Table 5.3.2 Ecobalance of the complete process using triticale corn in the distillery; data based on [hectare · a]

Emissions into air	CML2001, Greenhouse gas potential (GWP 100 years) kg CO2-equipollent		
Inorganic emissions into air			
Carbon dioxide	2148.2582	1087.1487	1061.1095
Nitrous oxide (Di-nitrogen-monoxide)	1212.3290	1203.4754	8.8537
Sulphur hexafluoride	0.0071	0.0071	<0.0001
Organic emissions into air (Group VOC)	0.0000		
Methane	251.2519	193.0688	58.1830
VOC (unspecific)	0.0429	0.0428	0.0002
Halogenic organic emissions into air	1.1545	0.5281	0.6264
Summation	**3613.04**	**2484.27**	**1128.77**
Emissions into air	**CML2001Acidification potential (AP); kg SO2-equipollent**		
Inorganic emissions into air			
Ammonia	14.2751	14.2710	0.0040
Ammonium	<0.0001	<0.0001	<0.0001
Ammonium nitrate	<0.0001	<0.0001	0.0001
Hydrogen chloride	0.0082	0.0058	0.0024
Hydrogen fluoride	0.0028	0.0020	0.0008
Sulphur dioxide	1.8282	0.9147	0.9135
Sulphuric acid	<0.0001	<0.0001	<0.0001
Hydrosulphide	0.3119	0.1262	0.1856
Nitrogen oxides	6.6528	5.8571	0.7957
Nitrate monoxide	0.9261	0.9261	<0.0001
Summation	**24.01**	**22.10**	**1.90**

Part 2 of table 5.3.2 Ecobalance of the complete process using triticale corn in the distillery; data based on [hectare · a]

Thus the higher energy yield of the fossil fed distillery process (fossil distillery 1) is obtained at the tense of a higher environmental pollution. With regard to the eutrophication, the partly autarkic fed process (autarkic distillery 1) produces a slightly lower eutrophication potential than the complete fossil fed process (fossil distillery 1). Extrapolated to the 1000 hectares of the model farm per year, ca. 180 kg of phosphate-equipollent can be saved. With CO_2 the difference is far more drastic. Regarding the greenhouse gas emissions, the partly autarkic process (autarkic distillery 1) saves more than 1.1t of CO_2-equipollents per hectare a year. If this saving is extrapolated to the complete area of 1000 hectares of the model farm, it is possible to save more than 1100t of CO_2-equipollents using the partly autarkic method of operation (autarkic distillery 1) compared to the fossil method of operation for the complete process (fossil distillery 1). As already mentioned above, the process using the autarkic-fed distillery (autarkic distillery 1) produces a lower acidification potential (SO_2-equipollent). With this method 1.9 kg of SO_2-equipollents can be saved per hectare per year. Thus compared to the fossil method of operation (fossil distillery 1), only ca. 92% of the SO_2-equipollents are produced. If this saving is extrapolated to the 1000 hectares, ca. 1.9t of SO_2-equipollents per year can be saved using the autarkic-fed distillery (autarkic In

spite of the higher energy yield of ca. 4%, of the fossil distillery process (fossil distillery 1) compared to the autarkic fed distillery process (autarkic distillery 1), on this background and remember the clearly worse ecobalance, the autarkic distillery process (autarkic distillery 1) is definitively the better and more sustainable process.

5.4. Distillery process using triticale corn and Maize silage to produce ethanol and usage of resulting stillage, triticale straw and remaining maize stillage in the biogas plant

Unlike to the distillery process described before, here two balances of distillery processes using triticale corn and maize silage to produce ethanol are described. The additional usage of maize silage in the distillery process should help to increase the ethanol yield of the sustainable agricultural process with crop rotation and the following distillery process. Fig. 5.4.1 shows the possible flow chart of such a combined process. Unlike starchy materials, maize silage is a lignocellulosic material and needs a different pre-treatment before it can be hydrolysed enzymatically to fermentable sugars. Additionally, the hydrolysis of the polysaccharides hemicellulose and cellulose needs different enzymes to the starch hydrolysis process. In a first step the maize silage can be reduced in size using a rotor-stator machine and adding tap water. To make sure that the dry substance of the resulting material does not decrease too much, after size reduction, the process stream is transferred to a standard stillage sieve. Here the surplus water is separated from the maize silage fibres. The collected water is recycled in the process and reused as process water for the rotor-stator machine. The dispersed maize silage is then transported to a high pressure vessel where it is pre-treated thermally. In the next step the maize silage is pre-hydrolysed using a cellulose-enzyme complex to decrease the viscosity of the slurry. For the ecobalance a 24 hour pre-hydrolysis step was calculated. After this 24-hour pre-hydrolysis, the starchy raw material triticale grits is added and hydrolysed for 2 hours by adding the amylolytic stargen^{TM} enzymes. Afterwards, the resulting mixture is fermented in a simultaneous saccharification and fermentation (SSF) process for 6 days. Analogue to the distillery process described in the previous chapter, the ethanol is separated from the complete fermented mash by distillation and dehydrated.

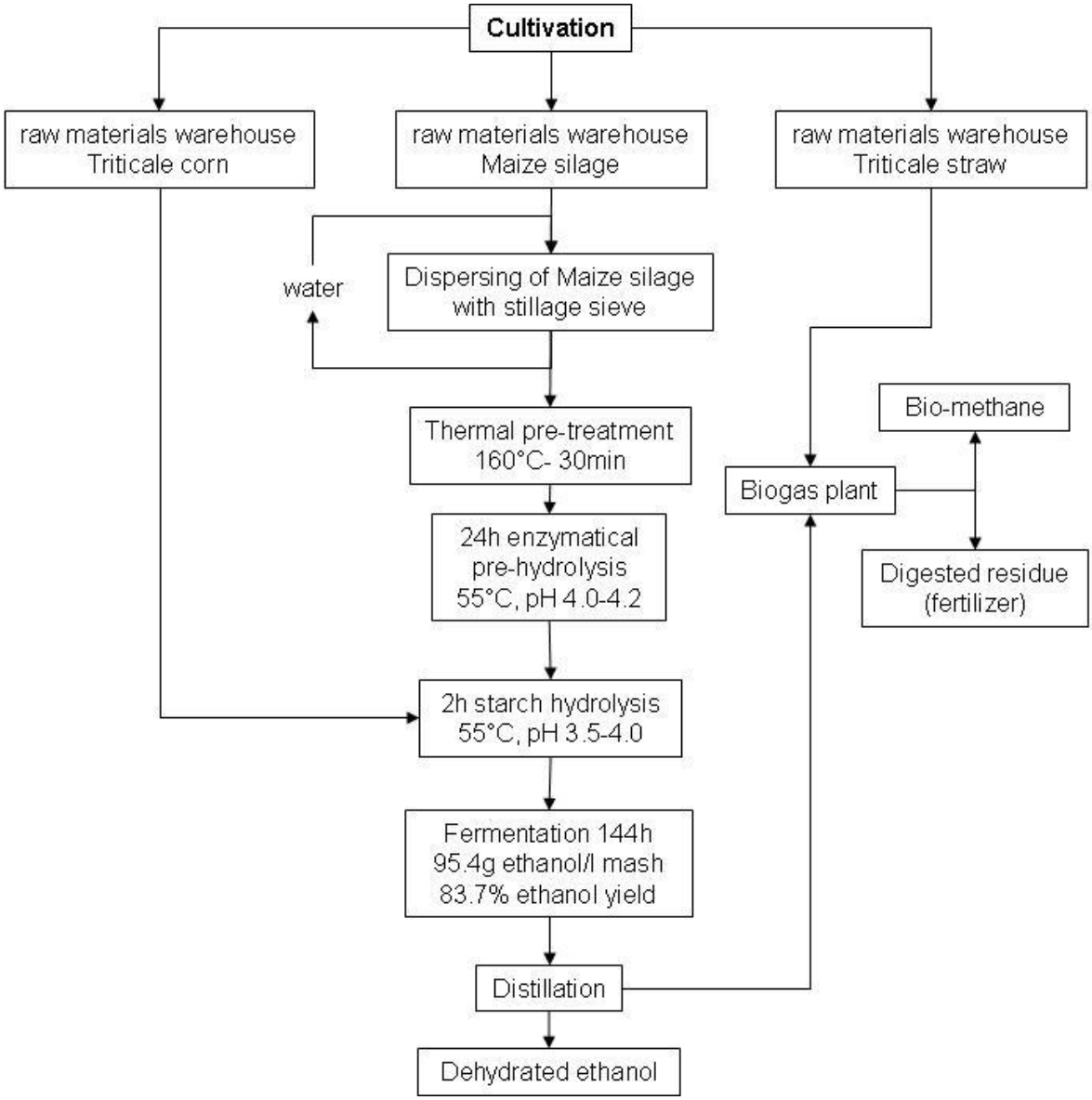


Fig. 5.4.1 Distillery process using triticale corn and maize silage to produce ethanol

The stillage resulting from the mash distillation process is mixed with the triticale straw and the remaining maize silage and is used as substrate for the biogas plant. As with the ecobalances in chapter 5.3, no manuring value of the digested substrate of the biogas plant is included in the balance. Also the same cultivation processes as described before is used for the balance. To compare what kind of energy supply is better for the distillery process two different methods are also modelled here. On the one hand the energy requirement of the distillery process is supplied using fossil fuels (fossil distillery 2), while on the other hand, the distillery process is fed using electricity and heat that is produced from a part of the bio-

methane using the BHKW (autarkic distillery 2). Analogous to the energy balance and ecobalance in the previous chapter, the production of the raw materials, the energy requirement for the material handling and the biogas plant are supplied using fossil fuels. All products that remain unused after the complete process (ethanol and remaining bio-methane) are balanced energetically.

	[MJ per hectare · a]		
	Autarkic distillery 2	Fossil distillery 2	Difference (fossil distillery- autarkic distillery)
Input			
Energy demand cultivation triticale	16 272.2	16 272.2	0
Energy demand cultivation maize	2 507.7	2 507.7	
Energy demand distillery	42 505.3	81 467.0	38 961.7
Sum energy input	**61 285.3**	**100 247.0**	**38 961.7**
Output			
Surplus electricity	4 419.2	0	-4 419.2
Ethanol	65 143.9	65 143.9	0
Methane	88 488.9	159 307.0	70 818.1
heat and electricity from BHKW for Distillery	42 505.3	0	-42 505.3
Sum energy output	**200 557.27**	**224 450.88**	**23 893.6**
Energy gain **(energy output – energy input)**	**139 272.0**	**124 203.9**	**-15 068.1**
Energy gain : Energy input	**2.27**	**1.24**	
Energy output : Energy input	**3.27**	**2.24**	

Table 5.4.1 Energy balance for the complete process using triticale corn and maize silage in the distillery

Table 5.4.1 shows the comparison of both methods of operation. The autarkic method of operation (autarkic distillery 2) only needs ca. 52% of energy sources compared to the fossil energy supply (fossil distillery 2). The reason for this is the high electricity requirement during the dispersing of the material. The fossil method of operation (fossil distillery 2) produces higher energy losses than the autarkic operation method (autarkic distillery 2), because the production of the electricity (here “Deutscher-Strom-Mix”) is less effective than the energy production using the BHKW. If the energy output of both processes is taken into consideration, the fossil fed process (fossil distillery 2) produces more energy products than the autarkic distillery process (autarkic distillery process 2). However, this higher

energy output is annulled in the complete energy balance because of the high energy requirement when the electricity and heat are produced from fossil fuels. Consequently, the fossil fed distillery process (fossil distillery 2) produces a lower energy gain than the autarkic fed distillery process (autarkic distillery 2). Only ca. 89% of the energy gain that is obtained using the autarkic energy supply can be yielded using the fossil method of operation (fossil distillery 2). When the relative and not the absolute data is regarded, which means that the energy gain of the complete process is related to the energy input, it can be seen that the autarkic fed distillery process (autarkic distillery 2) produces a much better result of 2.27: 1 (energy gain : energy input) than the fossil method of operation (fossil distillery 2). When the fossil energy supply method is used, only 1.24 MJ of energy can be gained from 1 MJ of fossil energy. This value is worse than the 1.32:1 (SENN und LUCÁ, 2002) for an industrial ethanol process because of the high energy requirement for the pre-treatment process of the cellulosic biomass.

Considering the ecobalance of both methods, the autarkic-fed distillery process (autarkic distillery 2) has a better eutrophication, greenhouse gas emission and better acidification than the complete process using a fossil fed distillery (fossil distillery 2). When working with the autarkic fed distillery (autarkic distillery 2), the eutrophication potential can be decreased to ca. 95% of the eutrophication potential of the process using the fossil fed distillery (fossil distillery 2). Extrapolating these results to the 1000 hectares of the model farm, the autarkic energy supply for the distillery (autarkic distillery 2) saves ca. 0.9 t of phosphate-equipollents per year compared to the fossil method of operation (fossil distillery 2).

If the greenhouse gas emissions are regarded, one simple result can be demonstrated. The only possible way to implement such a distillery process is to feed the distillery using a part of the energy products that are produced during the complete process. Thus, only an autarkic method of operation makes sense. When using this autarkic method of energy supply (autarkic distillery 2), only ca. 34% of greenhouse gases are produced compared to the fossil method of operation (fossil distillery 2). If this result is extrapolated to the size of 1000 hectares (size of the model farm), ca. 4900t of CO_2-equipollents can be saved per year using the autarkic fed distillery process (autarkic distillery 2). For the 1000 hectares of agricultural crop land the autarkic ethanol process (autarkic distillery 2)

produces ca. 254t of CO_2-equipollents. If this process is compared to a distillery process which is fed autarkic and only processes starchy materials (e.g. autarkic distillery 1), merely ca. 2% of CO_2-equipollents are produced because of the additional processing of the maize silage in the distillery.

	per hectare · a		
	Fossil distillery	**Autarkic distillery**	**Difference** (fossil distillery – autarkic distillery)
Emmissions into fresh water	**CML2001, eutrophication (EP); kg phosphate equipollent**		
Analysis values of emissions into fresh water			
Biochemical oxygen demand (BOD)	0.0003	0.0001	0.0002
Chemical oxygen demand (COD)	0.2714	0.0240	0.2474
Other	0.0059	0.0035	0.0024
Inorganic Emissions into fresh water			
Ammonia	0.0071	0.0071	<0.0001
Ammonium / Ammonia	0.1280	0.1240	0.0039
Nitrate	13.7235	13.7206	0.0029
Phosphate	0.4915	0.4906	0.0010
Nitrogen	<0.0001	<0.0001	<0.0001
Organically bound nitrogen	0.8524	0.8524	<0.0001
Organic Emissions into fresh water			
Hydrocarbon (unspecific)	0.0002	0.0001	0.0002
Methanol	0.0086	0.0086	<0.0001
Oils (unspecific)	0.0011	0,0005	0.0007
Xylene (Isomers; Dimethylbenzol)	0.0004	0.0004	<0.0001
Emissions into air			
Inorganic Emissions into air			
Ammonia	2.7265	2.7201	0.0064
Ammonium	<0.0001	<0.0001	<0.0001
Ammonium nitrate	<0.0001	<0.0001	<0.0001
Nitrogen oxides	1.6974	1.1136	0.5838
Nitrate monoxide	0.1772	0.1772	<0.0001
Summation	**20.09**	**19.24**	**0.85**
Emissions into air	**CML2001, Greenhouse gas potential (GWP 100 years) kg CO2-euqipollent**		
Inorganic Emissions into air			
Carbon dioxide	5730.1986	1113.0365	4617.1622
Nitrous oxide (Di-nitrogen-monoxide)	1273.9936	1232.1338	41.8598
Sulphur hexafluoride	0.0072	0.0072	<0.0001
Organic Emissions into air (Group VOC)			
Methane	415.5872	197.6664	217.9208
VOC (unspecific)	0.0451	0.0438	0.0013
Halogenic organic emissions into air	5.8902	0.5406	5.3496
Summation	**7425.72**	**2543.43**	**4882.29**

Table 5.4.2 Ecobalance of the complete process using triticale corn and maize silage in the distillery; data based on [hectare · a]

Emissions into air	**CML2001, Acidification potential (AP); kg SO2-equipollent**		
Inorganic Emissions into air			
Ammonia	14.6453	14.6109	0.0344
Ammonium	<0.0001	<0.0001	<0.0001
Ammonium nitrate	<0.0001	<0.0001	<0.0001
Hydrogen chloride	0.0264	0.0059	0.0205
Hydrogen fluoride	0.0089	0.0021	0.0069
Sulphur dioxide	6.7637	0.9365	5.8272
Sulphuric acid	<0.0001	<0.0001	<0.0001
Hydrosulphide	0.5770	0.1293	0.4478
Nitrogen oxides	9.1399	5.9965	3.1434
Nitrate monoxide	0.9482	0.9482	<0.0001
Summation	**32.11**	**22.63**	**9.48**

Part 2 of Table 5.4.2 Ecobalance of the complete process using triticale corn in the distillery; data based on [hectare · a]

Even the acidification potential of the fossil fed process (fossil distillery 2) is higher than that of the autarkic-fed process (autarkic distillery 2). Compared to the fossil method of operation (fossil distillery 2), the autarkic distillery process (autarkic distillery 2) only produces ca. 70% of the acidification potential. Extrapolated to the 1000 hectares, this denotes a higher production rate of ca. 9.5t of acidification equipollents for the fossil-fed distillery process (fossil distillery 2). Moreover, this process results in a lower energy gain. Considering that, a clear statement can be made. If a process is to be implemented using maize silage and triticale corn in one distillery, the only reasonable way to do so is to feed the distillery process autarkic. If such a process is fed with fossil fuels the resulting emission values are even worse than those of an ethanol plant adjoining a sugar refinery which produces dehydrated ethanol (SENN and LUCÁ, 2002) or the lifecycle of fossil fuels (SENN and LUCÁ, 2002). However, if it is autarkic-fed (e.g. autarkic distillery 2), the CO_2-greenhouse gas emission potential of this process is better than for the processes in the study by SENN (2002) despite the enormous energy requirement during the cellulose hydrolysis process.

5.5. Comparison of the ecobalances of the autarkic fed distillery processes

Because the autarkic-fed processes of the last two chapters (autarkic distillery 1 and autarkic distillery 2) clearly have better ecobalances than the fossil fed ones (fossil distillery 1 and fossil distillery 2), these two processes should now be compared with each other to find out the potential of an additional usage of maize silage in the distillery. As can be seen in table 5.5.1 the additional usage of maize silage in the distillery process results in a slight worse ecobalance.

	per hectare · a		
	Distillery only titicale	**Combined distillery**	**Difference** (combined distillery – distillery only triticale)
Emissions into fresh water **Analysis values of the emissions into fresh water**	**CML2001, eutrophication potential (EP); kg phosphate equipollent**		
Biochemical oxygen demand (BOD)	0.0001	0.0001	<0.0001
Chemical oxygen demand (COD)	0.0235	0.0240	-0.2474
Other	0.0034	0.0035	-0.0024
Inorganic emissions into fresh water			
Ammonia	0.0070	0.0071	0.0001
Ammonium / Ammonia	0.1211	0.1240	0.0028
Nitrate	13.4015	13.7206	0.3191
Phosphate	0.4792	0.4906	0.0114
Nitrate	0.0000	0.0000	0.0000
Organically bound nitrogen	0.8326	0.8524	0.0198
Organic emissions into fresh water			
Hydrocarbon into fresh water			
Hydrocarbon (unspecific)	0.0001	0.0001	<0.0001
Methanol	0.0084	0.0086	0.0002
Oils (unspecific)	0.0005	0.0005	<0.0001
Xylenel (Isomers; Dimethylbenzol)	0.0004	0.0004	<0.0001
Emissions into air			
Inorganic emissions into air			
Ammonia	2.6568	2.7201	0.0633
Ammonium	0.0000	0.0000	<0.0001
Ammonium nitrate	0.0000	0.0000	<0.0001
Nitrogen oxides	1.0877	1.1136	0.0259
Nitrogen monoxide	0.1731	0.1772	0.0041
Summation	**18.80**	**19.24**	**0.44**

Table 5.5.1 Comparison of the ecobalance of the different autarkic fed distillery processes

Emissions into air	CML2001, Greenhouse gas potential (GWP 100 years) kg CO2-equipollent		
Inorganic emissions into air			
Carbon dioxide	1087.1487	1113.0365	25.8878
Nitrous oxide (Di-nitrogen-monoxide)	1203.4754	1232.1338	28.6585
Sulphur hexafluoride	0.0071	0.0072	0.0002
Organic emissions into air (Group VOC)			
MethanE	193.0688	197.6664	4.5975
VOC (unspecific)	0.0428	0.0438	0.0010
Halogenic emissions into air	0.5281	0.5406	0.0126
Summation	**2484.27**	**2543.43**	**59.16**
Emissions into air	**CML2001, Acidification potential (AP); kg SO2-equipollent**		
Inorganic emissions into air			
Ammonia	14.2710	14.6109	0.3398
Ammonium	<0.0001	<0.0001	<0.0001
Ammonium nitrate	<0.0001	<0.0001	<0.0001
Hydrogen chloride	0.0058	0.0059	0.0001
Hydrogen fluoride	0.0020	0.0021	<0.0001
Sulphur dioxide	0.9147	0.9365	0.0218
Sulphuric acid	<0.0001	<0.0001	<0.0001
Hydrosulphide	0.1262	0.1293	0.0030
Nitrogen oxides	5.8571	5.9965	0.1395
Nitrate monoxide	0.9261	0.9482	0.0221
Summation	**22.10**	**22.63**	**0.53**

Part 2 of table 5.5.1 Comparison of the ecobalance of the different autarkic fed distillery processes

The reason for this fact is that the energy requirement for the pre-treatment of the cellulosic material (maize silage) results in a higher production of emissions. Although the combined process produces a higher energy output, this higher energy gain that could be achieved, is not vindicated compared to the higher emissions that are produced through the method of operation. If the values are extrapolated to the area of 1000 hectares farmland of the model farm, the additional usage of maize silage increases the eutrophication potential of ca. 0.44t phosphate equipollents per year. For the greenhouse gases ca. 59t more CO_2-equipollents are produced. Additionally, the acidification potential is increased to ca. 0.53t of SO_2-equipollents per year. The energy gain of the combined process is also lower. But if the hydrolysis processes of the cellulose material hydrolysis were to be improved, and whereby the energy requirement of such a process could be reduced, it should be possible to process maize silage as an additional raw material to produce ethanol. As a result the potential yield of ethanol produced in a sustainable distillery process should be improved.

5.6. Comparison of the ecobalances of the fossil fed distillery processes

Analogous to the autarkic fed processes, the combined distillery process using maize silage and triticale corn results in a higher energy output, but like the autarkic processes the clearly higher energy requirement during the maize silage pre-treatment results in a lower energy gain. In addition to this there is a clear increase in the CO_2-equipollents and an increase in the eutrophication and acidification potential respectively. If all the respective values are extrapolated to the area of 1000 hectares, it can be seen that the combined distillery process produces ca. 1.12t more phosphate equipollents. The difference with the CO_2 equipollents is much more dramatic. For that criterion the CO_2-equipollents are more than doubled. Consequently ca. 3812t more CO_2-equipollents are produced when the distillery process uses maize silage in addition to starch as raw materials for producing ethanol. As already mentioned above, the acidification potential increases with the combined distillery process. Extrapolated to the area of 1000 hectares of the model farm, an amount of ca. 8.10t of SO_2-equipollents are produced. Regarding all these results, one thing can easily be said. When feeding a distillery process using fossil fuels, the additional usage of maize silage for the production of ethanol is not a very good option. The process using starchy materials only and the further usage of the residues in a biogas process achieve much better results.

	per ha · a		
	Distillery only triticale	**Combined distillery**	**Difference** (combined distillery - distillery only triticale)
Emissions into fresh water **Analysis values of the emissions into fresh water**	**CML2001, Eutrophication potential (EP); kg phosphate equipollent**		
Biochemical oxygen demand (BOD)	0.0002	0.0003	0.0001
Chemical oxygen demand (COD)	0.0526	0.2714	0.2188
Other	0.0041	0.0059	0.0019
Inorganic emissions into fresh water			
Ammonia	0.0070	0.0071	0.0002
Ammonium / Ammonia	0.1216	0.1280	0.0064
Nitrate	13.4018	13.7235	0.3217
Phosphate	0.4794	0.4915	0.0122
Nitrate	<0.0001	<0.0001	<0.0001
Organically bound nitrogen	0.8326	0.8524	0.0198

Table 5.6.1 Comparison of the ecobalance of the fossil distillery processes

Organic emissions into fresh water			
Hydrocarbon into fresh water			
Hydrocarbon (unspecific)	0.0001	0.0002	0.0001
Methanol	0.0084	0.0086	0.0002
Oils (unspecific)	0.0007	0.0011	0.0004
Xylenel (Isomers; Dimethylbenzol)	0.0004	0.0004	<0.0001
Emissions into air			
Inorganic emissions into air			
Ammonia	2.6576	2.7265	0.0689
Ammonium	<0.0001	<0.0001	<0.0001
Ammonium nitrate	<0.0001	<0.0001	<0.0001
Nitrogen oxides	1.2355	1.6974	0.4619
Nitrogen monoxide	0.1731	0.1772	0.0041
Summation	**18.97**	**20.09**	**1.12**
Emissions into air	**CML2001, Greenhouse gas potential (GWP 100 Jahre) kg CO2 equipollent**		
Inorganic emissions into air			
Carbon dioxide	2148.2582	5730.1986	3581.9404
Nitrous oxide (Di-nitrogen-monoxide)	1212.3290	1273.9936	61.6646
Sulphur hexafluoride	0.0071	0.0072	0.0001
Organic emissions into air (Group VOC)			
MethanE	251.2519	415.5872	164.3353
VOC (unspecific)	0.0429	0.0451	0.0022
Halogenic emissions into air	1.1545	5.8902	4.7357
Summation	**3613.04**	**7425.72**	**3812.68**
Emissions into air	**CML2001, Acidification potential (AP); kg SO2 equipollent**		
Inorganic emissions into air			
Ammonia	14.2751	14.6453	0.3702
Ammonium	<0.0001	<0.0001	<0.0001
Ammonium nitrate	<0.0001	<0.0001	<0.0001
Hydrogen chloride	0.0082	0.0264	0.0182
Hydrogen fluoride	0.0028	0.0089	0.0061
Sulphur dioxide	1.8282	6.7637	4.9355
Sulphuric acid	<0.0001	<0.0001	<0.0001
Hydrosulphide	0.3119	0.5770	0.2652
Nitrogen oxides	6.6528	9.1399	2.4872
Nitrate monoxide	0.9261	0.9482	0.0221
Summation	**24.01**	**32.1095**	**8.10**

Part 2 Table 5.6.1 Comparison of the ecobalance of the fossil distillery processes

5.7. The utilization of modern heat recovery during the distillation of a distillery process using maize silage and triticale for ethanol production

Because the fossil distillery process, that uses maize silage as well as triticale for ethanol production, has a worse energy balance and ecobalence compared to an analogous autarkic distillery process, a fossil energy supplied distillery process using the latest technology is to be described and balanced below. Unlike the fossil fed distillery process of a rural distillery as described in chapter 5.3, for this distillery process heat recovery similar to that in huge distillery plants is calculated for the ecobalance. Additionally, the size reduction of the maize silage is

calculated using an economically operating hammer mill instead of the rotor-stator machine. The data for the hammer mill is taken from the Leitfaden Bioenergie 2005. There the energy requirement of a hammer mill crushing cellulosic materials is estimated at 0.8MJ/kg grinding stock. As already mentioned, the distilling section is calculated using heat recovery as described by KATZEN et al. (1999). With this technology the thermal energy demand is ca. 3.36MJ/kg dehydrated ethanol. During such a distillery process the heat is returned into the process several times to preheat colder process streams. By using this method of operation, such a process can save ca. 50% energy compared to the process that was balanced before.

	[MJ pro ha · a]		
	Autarkic distillery	Fossil distillery	Fossil distillery (hammer mill and heat recovery during distillation)
Input			
Energy demand cultivation triticale	16 272.2	16 272.2	16 272.2
Energy demand cultivation maize	2 507.7	2 507.7	2 507.7
Energy demand distillery	42 505.3	81 467.0	70 308.6
Sum energy input	**61 285.3**	**100 247.0**	**89 088.5**
Output			
Surplus electricity	44 19.21	0	0
Ethanol	65 143.9	65 143.9	65 143.9
Methane	88 488.9	159 307.0	159 307.0
heat and electricity from BHKW for distillery	42 505.3	0	0
Sum energy output	**200 557.27**	**224 450.88**	**224 450.88**
Energy gain (energy output – energy input)	**139 272.0**	**124 203.9**	**135 362.4**
Energy gain : Energy input	**2.27**	**1.24**	**1.52**
Energy output : Energy input	**3.27**	**2.24**	**2.52**

Table 5.7.1 Comparison of the distillery processes using maize silage and triticale to produce ethanol

Table 5.7.1 shows a survey of all three distillery processes that were balanced using maize silage and triticale in one process. The third column of the table shows the distillery process that is balanced using the new specifications. Using the modern heat recovery and the hammer mill makes it possible to achieve a

much higher energy gain compared to the distillery process using the rotor-stator system and the distillery process of a rural distillery. Consequently, it is possible to yield nearly the same energy gain as for an autarkic fed distillery process. Only ca. 3% losses have to be accepted. If it were possible to reduce the high energy requirement of the cellulase production (12 586.6MJ/ha) that results from the enormous amount of enzyme to be used during the process and the enormous thermal energy requirement of the pre-treatment step (15 164.0MJ/ha) by means of further research, the energy gain of such a distillery process could clearly be increased. Hence, such combined processes using starchy and cellulosic raw material for ethanol production could be profitable.

6. Discussion

6.1. Lignocellulosic biomass (corn silage)

For the pre-treatment of ethanol processes using cellulosic biomass (in this case corn silage) it could be shown that a temperature of 160°C and a dwell period of 50 to 60min result in the best digestibility of the biomass. Ca. 67 up to 80% (for some tests) of the cellulosic biomass could be hydrolysed within 48h of hydrolysis. Compared to other results (ÖHGREN et al., 2005) the results represent a very good basis considering they are already obtained after 48h of hydrolysis. ÖHGREN et al required 96h of enzymatic hydrolysis to achieve their results and worked at a dry substance concentration of 2%. In the discussed tests the best results are obtained at a dry substance of 5.27% and 7.2% respectively.

When using dispersed material for the tests, the digestibility of the corn silage could be increased. The reason for this is the size reduction of the particles and the consequently increased surface of the particles, thus the effect of the pre-treatment is increased. During the enzymatic hydrolysis of ca. 5.3% and 6.5% dry matter a hydrolysis of ca. 65 to 67% could be achieved within a hydrolysis period of 48h. The comparable tests using no further size reduced material resulted in ca. 55 to 59% enzyme hydrolysis using the same pre-treatment temperature and the same enzymatic hydrolysis time of 48h. If it is mentioned that in contrast to most other processes no further additives are added before pre-treatment and lower temperatures are used compared to the tests of ÖHGREN et al (2005), who used sulphur dioxide as catalyst at temperatures of 170 to 210°C for pre-treatment, the experiments represent good results. ÖHGREN et al. are able to hydrolyse ca. 60 to 67% of the glucane within 96h of hydrolysis.

In further tests the hydrolysis of the pre-treated biomass is extended. These tests are carried out to see if a longer enzymatic hydrolysis results in better glucane hydrolysis. Expectedly the longer enzymatic hydrolysis resulted in a better glucane hydrolysis. Up to 75% of glucane hydrolysis could be realized with dispersed corn

silage. Comparable results could be realized even using pre-treatment conditions previously considered not being optimal. After ca. 70 to 80 hours of enzymatic hydrolysis ca. 75% of the glucane is hydrolysed. The results are comparable to all the results of ÖHGREN et al. (2005). If it is taken in mind that ÖHGREN et al. (2005) always used higher temperatures and found out that high temperatures (up to 210°C) and short time in the pre-treatment (2 min instead of up to 10min) resulted in the highest glucose yields, these results show that there is no use for temperatures much higher than 160°C that are only used for short residence times. Such high temperatures represent a high usage of energy to heat up the biomass to temperatures of up to 210°C. Additionally vessels which work at this temperatures (ca. 210°C) and corresponding pressures of ca. 20bar are very expensive in investment. The higher the working pressures of a pre-treatment vessel the thicker the wall has to be made. Furthermore higher investments in valves and armatures and even the boiler have to be made. Working at lower temperatures of ca. 160°C it is easier to work with a cheaper vessel. Also boilers which typically can be found in local distilleries (up to 6bar) can be used for the pre-treatment vessel. As mentioned before it is possible to reach comparable results at lower pre-treatment temperatures to them using high temperatures for pre-treatment by using longer times for the pre-treatment step.

To solve the problem of dilution of the biomass during pre-treatment caused by the direct injection of the process steam, a vessel with indirect heating by use of a heating jacket is used. The most important thing that is to be pointed out using this vessel is that the dry substance before and after the pre-treatment is nearly the same. During the tests dry substances of ca. 15 to 20% can be realized after the pre-treatment step. If such high dry substances are used, realizing comparable hydrolysis rates like during the tests at max 7.5% dry substance, clearly higher ethanol yields should be obtained. Using the indirect heating and thereby resulting in a higher dry substance after the pre-treatment step a bit lower results compared to the tests using direct steam injection could be achieved. Glucane hydrolysis up to 60% is reached. Considering the fact that ÖHGREN et al. (2005) only used slurries at 2% dry substance for the enzymatic hydrolysis and obtained glucane hydrolysis rates up to ca. 80% of theoretical value, a 60% hydrolysis in a 15 to 20% dry substance slurry is very good. Because if 80% of the glucane of a 2% dry

substance slurry is hydrolysed only a small amount of glucose (in g/l) is liberated compared to 60% of a 15 to 20% dry matter. For 20% dry substance and 60% hydrolysis rate a glucose concentration ca. 60g/l is found in the slurry. If all the sugar is fermented by yeast with a 90% yield ca. 27g/l of ethanol would be yielded. This represents a result that is comparable to the results of ÖHGREN et al. (2006). Because even if the glucane of the corn silage is hydrolysed completely due to the relatively low dry substance (< ca. 20%) that can be handled during the hydrolysis and pre-treatment process only relatively low glucose concentrations can be achieved after a complete hydrolysis. For that reason also only ethanol concentrations of < 6%mas can be obtained in theory. Such ethanol concentrations in mashes can not be used in a profitable distillation. In such a process too much energy would be required to separate the ethanol. Therefore tests using both corn silage and starchy wheat cereals are carried out. In such processes it is possible to increase the potential concentration of glucose to a level that makes it possible to distil mashes at ethanol levels that are more profitable.

In the experiments different to all biofuels processes that are known, first corn sillage is pre-treated and pre-hydrolysed. After the pre-hydrolysis time the wheat cereals are added to increase the level of potential glucose. Enzymes hydrolysing granular starch are used to liberate the glucose from starch. Today this so called “stargen process” is used in some commercial distilleries to process starchy materials. One thing that is to be pointed out is that the stargen alpha-amylase works at the same temperature as the cellulase enzyme complex. So in parallel to the enzymatic cellulose hydrolysis also starch is digested.

A so called SSF (simultaneous saccharification and fermentation) process is used. The advantage of such kind of process is that during the complete fermentation process the glucose level is quite low (typically < 5%). Because of the relatively low glucose concentration and an adequate number of yeast cells that directly convert the liberated fermentable sugars to ethanol normally no infection organisms can be found in such mashes.

Ethanol yields of approximately 85% of the theoretical value are obtained in the experiments. On one hand this represents results that are comparable to normal

commercial distillery processes using starchy materials at high gravity processes (dry substance >30%) if only the ethanol yield at the end of the process and not the fermentation time is regarded. On the other hand those tests represent results that are better than the results of the work of ÖHGREN et al (2006) and ÖHGREN et al (2007). Additionally ethanol concentrations of up to 97g of ethanol/l despite of < 30g/l like in the tests of ÖHGREN et al (2006) and ÖHGREN et al (2007) or other typical so called 2nd generation ethanol processes are obtained. To make a comparison to industrial processes is very difficult because the process owning companies do not publish their results.

As can be seen in the work of ÖHGREN et al. (2006 and 2007) and in a technical bulletin of Genencor (2009) for the production of ethanol from cellulosic materials long fermentation times have to be used to achieve maximum ethanol yields. In the tests the process has been longer than in the both references but in difference to the references even after more than 100h of SSF ethanol is produced. An SSF process that takes about 7 days results in optimal ethanol yield. The most important thing that has to be pointed out is that the fermentation results in ethanol concentrations of up to 97g/l whereas other processes result in maximum produced ethanol concentrations of 30-35g/l. Therefore it can be said that the combined usage of starchy material and cellulosic biomass in one SSF process realises processes that can be profitable in distillation because of adequate ethanol concentrations in the mash. And if other fermentation organisms as xylose and arabinose fermenting yeasts or the new geobacillus bacteria TM 242 (TMO renewables) will be engineered for stable fermentation processes the ethanol production could be improved even more for the second generation ethanol process.

Additionally, no acid or other additives have been used for the pre-treatment during the process which means that neutralisation of the mash is not necessary before fermentation. And in contrast to other processes using additives the risk of sugar degradation products as furfurals, as potential fermentation inhibitors, is reduced.

6.2. Starchy materials

Additionally to the tests using lignocellulosic biomass for ethanol production further tests trying to improve the fermentation of starchy materials have been made. First of all the influence of nitrogen sources on accelerated fermentation speeds is tested using wheat as raw material. For those tests, mashes with no further amino nitrogen sources added are compared to mashes using additional nitrogen sources as urea (1000mg/l and 500mg/l) or stillage originating from an ethanol fermentation and distillation process. As a further method, to increase the concentration of amino nitrogen as yeast nutrition an acidic protease (GC 106, Genencor, Leiden Netherlands) is added to hydrolyse the proteins contained in the mash to usable amino acids.

Despite the results that could be realized in some studies (THOMAS and INGLEDEW, 1992; JONES and INGLEDEW, 1994; KOLOTHU-MANNIL et al., 1990; THOMAS and INGLEDEW, 1992 and THOMAS et al., 1993) no significant acceleration of the fermentation could be achieved by adding nitrogen to the mashes. All fermentation tests resulted in an ethanol production of ca. 80 to 90% of the theoretical value. All results already could be reached after 48h which represents a typical fermentation time of ca. 50h (RUSSEL, 2003) for distillery processes using the complete mash in the fermentation process. After further 24h of fermentation the ethanol yield that could be obtained is ca. 95% of the theoretical value for all experiments. This shows that the yeast was still vital after the 48h of fermentation. The enhancement of the fermentation time to 72 hours only has been done to check if the fermentation already had been completed. Nevertheless, one result that can be pointed out is that only the additional usage of high dosages of an amino nitrogen source (1000 mg urea/l) results in a slightly accelerated fermentation. But this is correlated with higher costs for additives. Using a complete wheat mash including all the resulting solids for a fermentation process the fermentation also is profitably finished after ca. 50 hours.

Additionally to the tests to try to accelerate the fermentation speed, experiments checking the influence of the dry substance on the ethanol yield are performed. One thing that is to be mentioned is that increasing the dry substance causes an

increase in the osmotic pressure on the yeast and thereby causes stress on the yeast. The higher the osmotic pressure caused by the liberated sugars the higher the stress for the yeast. So regarding the influence of the dry substance on the ethanol yield gives a good idea if an ethanol process can be run profitable.

Additionally to increasing the dry substance in some tests the fermentation temperature is set to a very high fermentation temperature of 40°C. Usually yeasts have a maximum growth temperature ca. 37-39°C (RUSSEL, 2003). Some special strains even can survive temperatures up to 43°C under ideal conditions without any further stress factors.

Regarding the ethanol yield that could be obtained after the tests, one clear statement can be made. The higher the dry substance the lower the ethanol yield after 72h results. The maximum dry substance that could result a > 90% ethanol yield of the theory in the laboratory tests is a dry substance of 30%. Using such a process an ethanol concentration >10% vol. can be realised producing a high theoretical ethanol yield. Further increasing the dry substance did not result in much higher ethanol concentrations than for the 25% dry substance. Consequently a decreased usage of the raw material has to be accepted.

If the tests are performed at the high fermentation temperature of 40°C it can be seen that the increasing osmotic stress combined with temperature stress induces a much stronger decrease regarding the ethanol yield. For the fermentations of maize as raw material those effects could be demonstrated much stronger. For those tests a dry substance of 25% and a fermentation temperature of 30°C represents the maximum dry substance that produces ethanol yields >90% during the 72h of fermentation. Increasing the fermentation temperature even for 20% dry substance to the limiting 40°C a considerable decrease to maximum 80% of theoretical ethanol yield is to be accepted.

For other fermentation tests a new enzyme system called stargen™ is used. This enzyme system works below the gelatinization temperature of the raw material and digests the granular starch. In normal processes this granular starch first is soaked with water to gelatinize the starch before the enzymes are able to digest

the starch to fermentable sugars. If the stargen™ enzyme system is used also non gelatinized starch can be digested by the enzymes to fermentable sugars. During such a process the viscosity of the mash is much lower than for mashes with gelatinizing starch. So a lot of energy can be saved during the stirring. Additionally usual enzyme systems work at higher temperatures than the stargen™ enzyme complex. So additionally to the lower stirring and pumping energy that is needed also less heating energy is to be used because the enzymes digest wheat or triticale starch at about 58°C. After a pre-hydrolysis of the starch using a special α-Amylase, which has a so-called starch binding domain to digest the granular starch, the mash is cooled down to fermentation temperature where a simultaneous saccharification and fermentation process (SSF) further digests the starch to fermentable sugars. In such a process all sugars are directly transferred to ethanol by the yeast as soon as they are liberated.

Analogous to the test using conventional enzyme systems the dry substance has a big influence on the ethanol yield at the end of the fermentation. The higher the dry substance the lower the ethanol yield at the end of the fermentation process. Similar to the tests shown above ethanol yields of ca. 90% from theory can be obtained. The only thing that is to be pointed out is the fermentation time. A longer process compared to the above discussed processes is to be accepted. The fermentation time takes longer than the ca. 50h. In the experiments comparable results to profitable distillation processes could be obtained within 65-72h for dry substances up to 30%. As for fermentation tests of LANTERO et al. (1991) using the stargen™ enzyme system, at the first stage (up to 20h) the yeast had an increased ethanol production rate of ca. 1.8-2.0g ethanol/(l*h). In the later phases the ethanol production rate decreases nearly proportional to the increased fermentation time. This effect can be explained because of the higher osmotic pressure in the beginning of the process that makes it easier for the yeast to transport the sugars into the cell. If the fermentation time is increased the sugar concentration is decreased because of the transformation of sugars to ethanol that is faster than the liberation of new fermentable sugars. Caused of this lower sugar concentration the osmotic pressure also decreases. Consequently the yeast cells slow down producing ethanol because lower amounts of fermentable sugars can be transported into the yeast cell. The special thing about SSF processes is that during, except of the first stage of the process when the yeast is adapting to the

media, the complete fermentation process the concentration of the fermentable sugars is very low at <2g/l. In normal distillery processes the starting sugar concentration is high so that the yeast first has to acclimatise to the high osmotic pressure. In SSF processes the osmotic pressure on the yeast is lower during the complete fermentation process. Furthermore, because of the low sugar content the risk of infections with bacteria is comparable small.

6.3. Ecobalance of different ethanol production concepts

6.3.1. Ecobalance for processes only using starchy material for ethanol production

In the ecobalance the results from the tests are used to calculate a system for sustainable ethanol production from biomass. The agricultural process for biomass production the starchy biomass (here triticale) and the energy maize is realised in crop rotation to ensure the sustainability. Two thirds of the farmland is used to cultivate the starchy biomass. On the surplus third of farmland the energy maize for sillage production is cultivated. In addition to the energy maize also triticale straw as a cellulosic energy source is recovered. To make sure that the distances for biomass transport are as short as possible the model farm owns about 1000 hectares of farmland. Additionally, all equipment that is needed to realise a sustainable ethanol production like the distillery, a biogas plant and a modern block heat and power plant (BHKW) are also owned by the model farm. To compare what kind of energy supply is the best for such an ethanol production two kinds of energy supply are calculated. In one scenario the distillery is supplied with heat and electricity using some biogas from the biogas production in the BHKW. This scenario represents a self sufficient process that needs no energy from fossil fuels for the distillery process. In a second scenario all the energy that is needed is supplied using fossil fuels. If only the starchy triticale is used to produce ethanol and all other recovered biomass and the stillage from the distillation process are used to produce biogas very high energy gain : energy input relations of more than 4 : 1 can be realised for both scenarios.

CO_2 emissions [kg/GJ]			
Fuel oil/Diesel	86.2	DE-Strommix	174
Natural gas	56	Fuel	85
Transport, Storage and addition of ethanol	0.4		
	Autarkic distillery [GJ/(ha · a)]	**Basis for substitution**	**Comparable CO2 emissions from fossil fuels** [kg CO_2/(ha · a)]
Surplus electricity	7.05	DE-Strommix	1 226.65
Ethanol	50.55	Fuel	4 297.09
Methane	116.29	Natural Gas	6 512.20
heat and electricity from BHKW for distillery	13.69	Fuel oil/Diesel	1 180.45
Sum energy output	**187.59**	**Sum of comparable fossil emissions**	**13 216.38**
Transport, storage and addition of ethanol			-20.22
Energy demand during complete process	-32.07		-2484.27
Energy gain	**155.52**	**Avoided CO_2 emissions [kg CO_2/(ha*a)]**	**10 711.89**
		Avoided CO_2 emissions [%]	**81.05**
	Fossil distillery [GJ/(ha · a)]	**Basis for substitution**	**Comparable CO_2 emissions from fossil fuels** [kg CO_2/(ha · a)]
Ethanol	50.55	Fuel	4 297.09
Methane	145.92	Natural Gas	8 171.70
Sum energy output	**196.48**	**Sum comparable emissions**	**12 468.79**
Transport, storage and addition of ethanol			-20.22
Energy demand during complete process	-37.06		-3 613.04
Energy gain	**159.42**	**Avoided CO_2 emissions [kg CO2/(ha*a)]**	**8 835.53**
		Avoided CO_2 emissions [%]	**70.86**

Table 6.3.1 The avoided CO_2 emissions for a distillery only using triticale for ethanol production

If the resulting energy products are used instead of energy sources produced from fossil fuels, and the avoidance of carbon dioxide (kg CO_2/GJ of energy) is calculated based on fossil energy production processes, ca. 81% (autarkic distillery) and ca. 71% (fossil distillery) of CO_2 can be saved running the distillery only on starchy material (table 6.3.1) and using all other cultivation products in the biogas production. Both results meet the demands of the EU- Renewable Energy

Directive (Biomasse-Nachhaltigkeitsverordnung - BioNachV) and correspond to 10 711.89 kg avoided CO_2 emissions per hectare and year for the autarkic distillery and 8 835.53 kg avoided CO_2 emissions per hectare and year for the fossil distillery.

6.3.2. Ecobalance for processes using corn silage and starchy material for ethanol production

In difference to the process described before if the ethanol process is fed with maize silage and starchy triticale to produce ethanol, a so called 2nd generation ethanol process, an energy gain to energy input relation of 2.27 : 1 (autarkic distillery) and 1.24 : 1 (fossil distillery) can be realized (table 6.3.2 and 6.3.3).

CO_2 emissions [kg/GJ]			
Fuel oil/Diesel	86.2	DE-Strommix	174
Natural gas	56	Fuel	85
Transport, Storage and addition of ethanol	0.4		
	Autarkic distillery [GJ/(ha · a)]	**Basis for substitution**	**Comparable CO2 emissions from fossil fuels** [kg CO_2/(ha · a)]
Surplus electricity	4.42	DE-Strommix	768.94
Ethanol	65.14	Fuel	5 537.23
Methane	88.49	Natural Gas	4 955.38
heat and electricity from BHKW for distillery	42.51	Fuel oil/Diesel	3 663.96
Sum energy output	**200.56**	**Sum comparable emissions**	**14 925.51**
Transport, storage and addition of ethanol			-26.06
Energy demand during complete process	-61.29		-2 543.43
		Avoided CO_2 emissions [kg CO_2/(ha*a)]	**12 356.02**
		Avoided CO_2 emissions [%]	**82.78**

Table 6.3.2 The avoided CO_2 emissions for a distillery using triticale and maize sillage for ethanol production (part 1)

This still represents relatively good results compared to commercial distillery processes.

For the fossil distillery using maize silage and starchy triticale to produce ethanol a positive CO_2 balance can be obtained only because of the production of methane from straw and stillage. If only ethanol is produced more CO_2 would be produced compared to normal fossil energy processes because of the high energy demand during the distillery process (100.25 GJ/[ha · a]). One interesting result is that supplying such a process using renewable energy (autarkic distillery) makes it possible to realise a CO_2 avoiding potential of ca. 83% instead of the ca 48% for the fossil supplied process, which easily meets the demand of the Biomass Sustainability Ordinance. Here the higher ethanol yield that is obtained during the process compared to the process that only is using starchy materials to produce ethanol leads to that really good result. So if a process using cellulosic and starchy material is to be run in a distillery the only expedient way to do that is to supply the process using renewable energy sources.

	Fossil distillery [GJ/(ha · a)]	**Basis for substitution**	**Comparable CO_2 emissions from fossil fuels** [kg CO_2/(ha · a)]
Ethanol	65.14	Fuel	5 537.23
Methane	159.31	Natural Gas	8 921.19
Sum energy output	**224.45**	**Sum comparable emissions**	**14 458.42**
Transport, storage and addition of ethanol			-26.06
Energy demand during complete process	-100.25		-7 425.72
Energy gain	**124.20**	**Avoided CO_2 emissions [kg CO_2/(ha*a)]**	**7 006.65**
		Avoided CO_2 emissions [%]	**48.46**

Table 6.3.3 The avoided CO_2 emissions for a distillery using triticale and maize sillage for ethanol production (part 2)

7. Summary

In this thesis, a process was realized that uses starchy raw material (triticale) as well as lignocellulosic biomass (corn silage) in one ethanol production process. In contrast to other so called 2nd generation ethanol processes, which only use lignocellulosic material, the problem of the very low potential ethanol concentration (<max 6%mas) in such mashes is avoided. By the addition of starchy material to the pre-treated and pre-hydrolysed lignocellulosic biomass, it is possible to produce up to 96g ethanol/l in the mash within 144h of fermentation at ethanol yields of at least 84%. If such a process would be performed longer, the ethanol yield should approach the yields of current starch only processes.

This process not only produces ethanol concentrations that can be distilled profitably, it also has an ecobalance very positive. If the resulting stillage is used to produce biogas and part of the biogas is then used in a combined heat and power plant to supply the distillery process with heat and electricity, a self-sustaining distillery process can be realised. Using such self-sustaining ethanol production process about 83% of CO_2-emissions (CO_2-equipollents) can be avoided associated with the production of surplus energy via the energy products ethanol [ca. 65,14GJ/(ha · a)] and surplus non-purified biogas [ca. 88,49GJ/(ha · a)]. Even if such a process would be run using fossil fuels to produce heat and electricity (“Deutscher-Strommix”) about 48% of the CO_2-emissions (CO_2-equipollents) could be avoided. Such a process already fulfiles the demands of the “Biomasse-Nachhaltigkeitsverordnung” (BioNachV) [30% CO_2 reduction potential; and from 1st of January 2011 minimum 40% CO_2 reduction potential] that finally will be enacted in 2010.

In this thesis, also the basic so-called 1st generation process using only starchy material for ethanol production is eco-balanced. Such a process that uses stillage as well as recoverable straw and corn silage to produce biogas and is additionally supplied with heat and electricity from fossil sources, can reduce the CO_2-emissions to about 71%. If this number is expressed as CO_2-equipollents ca. 8.8t CO_2/(ha · a) can be avoided. This can be realised because 50.55GJ/(ha · a) ethanol and non-purified biogas [145.92GJ/(ha · a)] can be obtained. If such a process is run self-sustaining using part of the biogas to supply the distillery

process with heat and electricity, a considerable higher CO_2-reduction of about 81% can be obtained. This excellent result corresponds to an avoidance of 13.2t CO_2/(ha · a). Also 50.55GJ/(ha · a) ethanol and non-purified 116.29GJ/(ha · a) biogas can be obtained as energy products.

The data presented are based on laboratory tests and measurements in a pilot plant distillery without any energy recovery. If such processes are implemented in industrial production plants that can profitably realise energy recovery, further improvements of CO_2-reduction combined with a lower demand for utilities (steam, electricity, compressed air, etc.) should be possible. In addition, if other fermentation organisms like xylose and arabinose fermenting yeasts or the new geobacillus bacteria TM 242 (TMO renewables) are adopted to a stable fermentation process, the ethanol production could be improved even further.

Apart from the process using starchy and lignocellulosic raw material for ethanol production, further improvements of the so-called 1st generation process were explored in laboratory tests. The impact of enhanced yeast nitrogen supply as well as increased fermentation temperature for higher biochemical reaction rates was assessed. But in contrast to results from other studies, these measures did not result in positive effects on fermentation speed.

The only measure that could improve the 1st generation ethanol process by saving energy during the mash process is the application of the stargen^{TM} enzyme system. This enzyme system helps to digest granular starch without any gelatinisation before enzymatic starch hydrolysis. The disadvantage is, that the process takes more time to completely convert sugar to ethanol. Additionally more enzyme corresponding to higher enzyme costs is required.

A way to optimise the 1st generation ethanol processes, would be to separate the bran from the rest of the grain. In this way, only the starchy material that can be digested to fermentable sugars needs to be processed in the ethanol production process resulting in a lower energy demand during mashing and distillation, because of lower viscosity in the mash and less material that needs to be heated. The dry bran could be sold as by-product or could be used to produce biogas or could be burned to produce heat without any drying.

8. Zusammenfassung

In dieser Dissertation wurde ein Prozess realisiert, in dem stärkehaltige (Tritikale) und lignocellulosehaltige (Maissilage) Biomasse in einem Prozess zur Bioethanolerzeugung genutzt werden. Im Unterschied zu sonstigen so genannten 2. Generation Ethanolprozessen, welche nur lignocellulosehaltige Biomasse nutzen, wurde hier die Problematik der potenziell sehr geringen Ethanolkonzentrationen (< maximal 6%mas) in den erzeugten Maischen umgangen. Durch die zusätzliche Zugabe von stärkehaltigem Rohstoff zum bereits vorbehandelten und vorhydrolysierten lignocellulosehaltigen Material konnten in einer 144stündigen Fermentation Maischen mit bis zu 96g Ethanol/l mit einer Ethanolausbeute bis zu 84% erzeugt werden. Würde ein solcher Prozess länger geführt, wäre eine Erhöhung der Ethanolausbeute auf ein vergleichbares Niveau zu heutigen stärkebasierten Industrieprozessen möglich.

Neben dem sehr erfreulichen Effekt, dass die im dargestellten Prozess erzeugten Maischen mit profitabel zu destillierenden Alkoholkonzentrationen erzeugt werden können, lässt sich mit solch einem Prozess auch eine sehr positive Ökobilanz erzielen. Wird die im Prozess bei der Destillation erhaltene Schlempe zur Biogasproduktion eingesetzt und ein Teilstrom des erzeugten Biogases mittels eines BHKW zur Erzeugung von Prozesswärme und Strom für die Brennerei genutzt, ist es möglich einen autark versorgten Brennereibetrieb zu verwirklichen. Wird solch ein autark versorgter Ethanolprozess genutzt, so ergibt sich dadurch im Vergleich zur Nutzung fossiler Energieträger ein CO_2-Vermeidungspotential (CO_2-Equivalente) von ca. 83%, welches mit der Erzeugung von überschüssiger Energie in Form der Energieprodukte Ethanol [ca. 65,14GJ/(ha · a)] und noch unaufgereinigtem Biogas [ca. 88.49GJ/(ha · a)] gekoppelt ist. Wird dieser Prozess mit fossilen Ressourcen zur Erzeugung der Prozessenergie wie Strom (Deutscher-Strommix) und Prozesswärme versorgt, so kann ein CO_2-Vermeidungspotential (CO_2-Equivalente) von ca. 48% erzielt werden. Prozesse wie diese erfüllen bereits die Erfordernisse der Biomassenachhaltigkeitsverordnung (BioNachV), welche 2010 in Kraft tritt und sofort eine 30%ige CO_2-Reduktion und ab 1. Januar 2011 eine Mindestreduktion von 40% fordert.

Neben diesen Prozessen wurden in dieser Arbeit aber auch sogenannte Prozesse der 1. Generation, welche nur stärkehaltige Materialien zur Ethanolerzeugung

nutzen bilanziert. Wird bei diesen Prozessen sowohl eine fossile Energieerzeugung angenommen und aus der Schlempe als auch aus allem nachhaltig bergbaren Getreidestroh und der Maissilage Biogas erzeugt, so kann eine CO_2-Reduktion (CO_2-Equivallente) von ca. 71% erzielt werden. Wird dieses Vermeidungspotenzial als CO_2-Äquivalente ausgedrückt, so können ca. 8,8t CO_2/(ha · a) vermieden werden. Dies wird durch die Erzeugung von 50,55GJ/(ha · a) an Ethanol und 145,92GJ/(ha · a) an unaufgereinigtem Biogas möglich. Wird solch ein Prozess autark versorgt, was bedeutet, dass ein Teilstrom vom erzeugten Biogas den Brennereiprozess mit Prozesswärme und Strom versorgt, so kann eine deutlich höhere CO_2-Vermeidung von ca. 81% erzielt werden. Dieses exzellente Ergebnis korrespondiert mit einer CO_2-Vermeidung von ca. 13,2t CO_2(ha · a) und einer Erzeugung von 50,55GJ/(ha · a) an Ethanol und 116.29GJ/(ha · a) unaufgereinigtem Biogas als Energieprodukte.

Die oben diskutierten Daten basieren alle auf Untersuchungen im Labormaßstab sowie Messungen in der Versuchsbrennerei, welche ohne Energierückgewinnung arbeiten. Würde solche Prozesse jedoch im industriellen Maßstab genutzt, wo Energierückgewinnungssysteme wirtschaftlich eingesetzt werden, ließe sich der CO_2-Ausstoß weiter verringern und würde der Bedarf an Betriebsmitteln (Dampf, Strom, Druckluft, usw.) weiter sinken. Durch Einsatz kürzlich kommerziell verfügbarer Fermentationsorganismen, wie xylose- und arabinosefermentierende Hefen oder dem neuen Genobakterium TM 242 (der Firma TMO Renewables) kann der Ethanolerzeugungsprozess weiter verbessert werden.

Neben der Ethanolerzeugung aus stärke- und cellulosehaltigen Rohmaterialien wurden auch Versuche zur Verbesserung sogenannter Ethanolprozesse der 1. Generation durchgeführt. Um diese zu optimieren, wurden sowohl Strategien basierend auf einer verbesserten Stickstoffversorgung der Hefe als auch einer durch Temperaturerhöhung forcierten biochemischen Reaktionsgeschwindigkeit der Hefe, untersucht. Jedoch erzielten diese Techniken im Unterschied zu den Ergebnissen anderer Studien keine positiven Effekte auf die Fermentationsgeschwindigkeit.

Als vorteilhaft erwies sich jedoch die Verwendung des „Stargen^{TM}“-Enzymsystems, welches die Möglichkeit bietet granuläre Stärke ohne vorherige Verkleisterung zu hydrolysieren. Mit diesem System lässt sich der Energiebedarf beim Maischen deutlich senken. Dieses System besitzt jedoch den Nachteil, dass der

Fermentationsprozess länger als bei herkömmlichen Systemen dauert und ein höherer Enzymeinsatz erforderlich ist.

Die Separation der Kleie vom Restkorn wäre ein möglicher Weg Ethanolprozesse der 1. Generation zu verbessern. Wird dies realisiert, muss im Brennereiprozess nur noch stärkehaltiges Material, welches enzymatisch hydrolysiert werden kann, verarbeitet werden. Dies würde aufgrund einer geringeren Viskosität der Maische und einem geringeren Massefluss im Prozess den Energieaufwand während des Maischens und der Destillation reduzieren. Die abgetrennte, trockene Kleie kann dann einerseits als Nebenprodukt verkauft oder andererseits zur Erzeugung von Biogas oder Prozesswärme durch Verbrennung genutzt werden.

1. The influence of the pre-treatment conditions on the enzymatic hydrolysis of corn silage using direct heating for pre-treatment

pretreatment temperature [°C]	dwell period [min]	DS [%]	yield of hydrolysed glucan after 48h [%]
135	15	9,5	29,4
135	20	9,5	40,2
140	15	9,5	29,0
140	20	9,5	34,3
140	30	9,5	29,8
140	40	9,5	36,6
140	50	9,5	41,0
140	60	9,5	39,2
145	15	9,5	37,1
145	20	9,5	37,6
145	30	9,5	40,7
145	40	9,5	55,2
145	50	9,5	35,8
145	60	7,5	43,3
150	15	9,5	39,7
150	20	9,5	35,8
150	30	9,5	38,7
150	40	9,5	42,7
150	50	9,5	38,8
150	60	9,5	33,5
155	40	9,5	57,7
160	30	6,5	55,4
160	50	5,27	59,1
160	60	7,2	68,0
160	70	7	46,2
160	80	10	36,9
160	90	9,5	35,3

duration of test [h]	135-15				135-20				140-15				140-20			
	HPLC Versuch 1	Ausbeute [%]	HPLC Versuch 2	Ausbeute [%]	HPLC Versuch 1	Ausbeute [%]	HPLC Versuch 2	Ausbeute [%]	HPLC Versuch 1	Ausbeute [%]	HPLC Versuch 2	Ausbeute [%]	HPLC Versuch 1	Ausbeute [%]	HPLC Versuch 2	Ausbeute [%]
2	3,90	7,52	5,00	9,65	4,43	8,55	5,22	10,08	4,00	7,72	5,20	10,03	5,62	10,84	5,55	10,72
4	5,78	11,15	7,33	14,14	6,42	12,38	5,58	10,77	5,22	10,07	6,24	12,05	8,13	15,68	6,88	13,27
6	6,29	12,14	8,46	16,32	7,59	14,65	6,54	12,63	6,80	13,12	7,56	14,58	9,16	17,67	8,28	15,97
22	9,37	18,09	14,18	27,36	9,48	18,30	10,25	19,77	10,65	20,55	12,19	23,52	11,57	22,32	11,65	22,49
24	9,71	18,73	14,67	28,31	12,98	25,05	11,05	21,33	11,27	21,75	12,49	24,10	13,22	25,51	12,09	23,33
26	10,56	20,38	14,58	28,13	12,06	23,28	11,66	22,50	12,23	23,59	14,17	27,35	14,80	28,56	11,11	21,44
28	12,03	23,21	14,34	27,67	14,05	27,11	7,69	14,84	12,66	24,42	13,53	26,11	14,60	28,18	13,62	26,28
46	14,95	28,84	20,90	40,33	19,56	37,74	14,98	28,90	13,55	26,15	15,05	29,05	20,32	39,21	17,11	33,02
48	13,72	26,48	20,82	40,17	17,41	33,60	15,25	29,42	11,45	22,10	15,03	29,00	17,05	32,90	17,77	34,29
50	23,69	45,70	24,43	47,14	28,16	54,33	20,13	38,84	23,02	44,41	17,55	33,87	29,37	56,67	23,22	44,81
94	26,39	50,91	25,39	48,99	29,35	56,62	19,81	38,23	26,91	51,93	18,21	35,14	32,91	63,50	22,56	43,53
96	30,67	59,17	25,56	49,32	31,21	60,22	18,52	35,73	26,86	51,83	18,50	35,69	37,08	71,54	22,24	42,92

In experiment 1: for each test 2nd enzyme dosage after 48h
Maximal gucose content in mash 51,829 g/l

140-15

duration of test [h]	Glucose		Glucose corrected (without enzyme)		Glucose corrected to 400g mash		Hydrolysed glucane [%]	Xylose		Xylose corrected (without enzyme)		Xylose corrected to 400g mash		Arabinose		Arabinose corrected to 400g mash	
	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2		Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2
2	7,280	7,660	5,560	5,940	2,224	2,376	9,86	6,075	6,935	5,199	6,059	2,080	2,424	0,585	0,635	0,234	0,254
4	9,135	9,410	7,415	7,690	2,966	3,076	13,34	7,265	8,270	6,389	7,394	2,556	2,958	0,640	0,670	0,256	0,268
6	10,225	10,145	8,505	8,425	3,402	3,370	15,10	7,960	8,700	7,084	7,824	2,834	3,130	0,655	0,690	0,262	0,276
22	16,680	15,945	14,960	14,225	5,984	5,690	26,92	10,805	11,860	9,929	10,984	3,972	4,394	0,850	0,850	0,340	0,340
24	17,335	16,745	15,615	15,025	6,246	6,010	28,32	10,990	12,280	10,114	11,404	4,046	4,562	0,855	0,870	0,342	0,348
26	18,080	16,365	16,360	14,645	6,544	5,858	28,67	11,295	11,745	10,419	10,869	4,168	4,348	0,870	0,835	0,348	0,334
28	18,495	17,170	16,775	15,450	6,710	6,180	29,85	11,465	12,100	10,589	11,224	4,236	4,490	0,855	0,880	0,342	0,352
30	17,800	16,730	16,080	15,010	6,432	6,004	28,76	10,855	11,725	9,979	10,849	3,992	4,340	0,840	0,850	0,336	0,340
46	21,380	20,590	19,660	18,870	7,864	7,548	35,93	11,980	13,040	11,104	12,164	4,442	4,866	0,925	0,940	0,370	0,376
48	22,140	21,025	20,420	19,305	8,168	7,722	37,09	12,280	13,155	11,404	12,279	4,562	4,912	0,925	0,920	0,370	0,368

maximum yield from 38g DS [g]	hydro correction	
18,2	20,7	Glucose
10,0	11,1	Xylose
1,5	1,7	Arabinose

145-20

duration of test [h]	Glucose		Glucose corrected (without enzyme)		Glucose corrected to 400g mash		Hydrolysed glucane [%]	Xylose		Xylose corrected (without enzyme)		Xylose corrected to 400g mash		Arabinose		Arabinose corrected to 400g mash	
	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2		Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2
2	7,430	8,065	5,069	5,704	2,028	2,282	10,39	5,005	4,800	4,129	3,924	1,652	1,570	0,645	0,590	0,258	0,236
4	9,345	9,610	6,984	7,249	2,794	2,900	13,73	6,065	5,530	5,189	4,654	2,076	1,862	0,695	0,650	0,278	0,260
6	10,470	10,600	8,109	8,239	3,244	3,296	15,77	6,525	5,845	5,649	4,969	2,260	1,988	0,730	0,670	0,292	0,268
22	16,450	15,530	14,089	13,169	5,636	5,268	26,30	8,900	7,765	8,024	6,889	3,210	2,756	0,905	0,810	0,362	0,324
24	17,475	17,025	15,114	14,664	6,046	5,866	28,73	9,385	8,410	8,509	7,534	3,404	3,014	0,970	0,895	0,388	0,358
26	17,695	18,170	15,334	15,809	6,134	6,324	30,04	8,710	9,780	7,834	8,904	3,134	3,562	0,910	0,960	0,364	0,384
28	17,915	17,920	15,554	15,559	6,222	6,224	30,02	9,430	8,770	8,554	7,894	3,422	3,158	0,960	0,880	0,384	0,352
30	17,470	16,160	15,109	13,799	6,044	5,520	27,89	8,980	7,715	8,104	6,839	3,242	2,736	0,880	0,785	0,352	0,314
46	21,725	21,535	19,364	19,174	7,746	7,670	37,18	10,565	9,710	9,689	8,834	3,876	3,534	1,020	0,965	0,408	0,386
48	21,880	21,835	19,519	19,474	7,808	7,790	37,62	10,595	9,775	9,719	8,899	3,888	3,560	1,020	0,940	0,408	0,376

maximum yield from 38g DS [g]	hydro correction	
18,2	20,7	Glucose
10,0	11,1	Xylose
1,5	1,7	Arabinose

145-30

duration of test [h]	Glucose		Glucose corrected (without enzyme)		Glucose corrected to 400g mash		Hydrolysed glucane [%]	Xylose		Xylose corrected (without enzyme)		Xylose corrected to 400g mash		Arabinose		Arabinose corrected to 400g mash	
	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2		Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2
2	9,370	8,915	7,009	6,554	2,804	2,622	13,08	6,605	5,525	5,729	4,649	2,292	1,860	0,675	0,595	0,270	0,238
4	11,175	10,625	8,814	8,264	3,526	3,306	16,48	7,595	6,295	6,719	5,419	2,688	2,168	0,670	0,585	0,268	0,234
6	11,930	14,250	9,569	11,889	3,828	4,756	20,70	7,905	8,285	7,029	7,409	2,812	2,964	0,675	0,760	0,270	0,304
22	18,365	16,720	16,004	14,359	6,402	5,744	29,29	11,075	8,920	10,199	8,044	4,080	3,218	0,860	0,770	0,344	0,308
24	19,275	18,790	16,914	16,429	6,766	6,572	32,17	11,420	9,830	10,544	8,954	4,218	3,582	0,900	0,810	0,360	0,324
26	19,665	20,520	17,304	18,159	6,922	7,264	34,21	11,495	10,795	10,619	9,919	4,248	3,968	0,905	0,860	0,362	0,344
28	19,275	18,865	16,914	16,504	6,766	6,602	32,24	11,115	9,760	10,239	8,884	4,096	3,554	0,870	0,750	0,348	0,300
30	19,615	18,845	17,254	16,484	6,902	6,594	32,55	11,220	9,540	10,344	8,664	4,138	3,466	0,895	0,805	0,358	0,322
46	23,590	21,930	21,229	19,569	8,492	7,828	39,36	12,570	10,480	11,694	9,604	4,678	3,842	0,925	0,850	0,370	0,340
48	23,690	23,200	21,329	20,839	8,532	8,336	40,68	12,435	10,895	11,559	10,019	4,624	4,008	0,895	0,830	0,358	0,332

maximum yield from 38g DS [g]	hydro correction	
6,4	7,2	Glucose
5,5	6,1	Xylose
2,2	2,4	Arabinose

145-40

duration of test [h]	Glucose		Glucose corrected (without enzyme)		Glucose corrected to 400g mash		Hydrolysed glucane [%]	Xylose		Xylose corrected (without enzyme)		Xylose corrected to 400g mash		Arabinose		Arabinose corrected to 400g mash	
	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2		Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2
2	8,495	8,015	6,134	5,654	2,454	2,262	11,37	7,515	6,275	6,639	5,399	2,656	2,160	0,575	0,485	0,230	0,194
4	9,485	9,985	7,124	7,624	2,850	3,050	14,23	8,235	7,750	7,359	6,874	2,944	2,750	0,580	0,580	0,232	0,232
6	10,685	12,240	8,324	9,879	3,330	3,952	17,56	9,125	9,300	8,249	8,424	3,300	3,370	0,620	0,660	0,248	0,264
22	15,185	16,040	12,824	13,679	5,130	5,472	25,57	11,365	10,925	10,489	10,049	4,196	4,020	0,745	0,730	0,298	0,292
24	17,380	16,980	15,019	14,619	6,008	5,848	28,59	12,710	11,290	11,834	10,414	4,734	4,166	0,815	0,740	0,326	0,296
26	17,020	16,250	14,659	13,889	5,864	5,556	27,54	11,135	11,715	10,259	10,839	4,104	4,336	0,755	0,740	0,302	0,296
28	16,875	17,185	14,514	14,824	5,806	5,930	28,30	12,020	11,165	11,144	10,289	4,458	4,116	0,710	0,705	0,284	0,282
30	17,245	16,275	14,884	13,914	5,954	5,566	27,78	12,010	10,440	11,134	9,564	4,454	3,826	0,720	0,655	0,288	0,262
46	20,625	20,305	18,264	17,944	7,306	7,178	34,93	12,880	11,770	12,004	10,894	4,802	4,358	0,840	0,760	0,336	0,304
48	22,425	39,480	20,064	37,119	8,026	14,848	55,17	13,795	12,720	12,919	11,844	5,168	4,738	0,850	1,355	0,340	0,542

maximum yield from 38g DS [g]	hydro correction	
18,2	20,7	Glucose
10,0	11,1	Xylose
1,5	1,7	Arabinose

145-50

duration of test [h]	Glucose		Glucose corrected (without enzyme)		Glucose corrected to 400g mash		Hydrolysed glucane [%]	Xylose		Xylose corrected (without enzyme)		Xylose corrected to 400g mash		Arabinose		Arabinose corrected to 400g mash	
	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2		Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2
18,5	14,335	13,045	11,974	10,684	4,790	4,274	21,86	9,945	8,375	9,069	7,499	3,628	3,000	0,640	0,610	0,256	0,244
21	13,305	12,875	10,944	10,514	4,378	4,206	20,70	8,895	8,210	8,019	7,334	3,208	2,934	0,565	0,535	0,226	0,214
24	14,095	14,290	11,734	11,929	4,694	4,772	22,83	9,120	8,735	8,244	7,859	3,298	3,144	0,595	0,940	0,238	0,376
43,5	19,075	19,835	16,714	17,474	6,686	6,990	32,98	10,725	10,590	9,849	9,714	3,940	3,886	0,715	0,695	0,286	0,278
46	20,560	19,770	18,199	17,409	7,280	6,964	34,35	11,430	10,385	10,554	9,509	4,222	3,804	0,715	0,680	0,286	0,272
48	21,935	19,935	19,574	17,574	7,830	7,030	35,84	12,090	10,480	11,214	9,604	4,486	3,842	0,735	0,660	0,294	0,264

maximum yield from 38g DS [g]	hydro correction	
18,2	20,7	Glucose
10,0	11,1	Xylose
1,5	1,7	Arabinose

145-60

duration of test [h]	Glucose		Glucose corrected (without enzyme)		Glucose corrected to 400g mash		Hydrolysed glucane [%]	Xylose		Xylose corrected (without enzyme)		Xylose corrected to 400g mash		Arabinose		Arabinose corrected to 400g mash	
	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2		Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2
18,5	11,095	11,475	8,734	9,114	3,494	3,646	21,81	6,465	6,740	5,589	5,864	2,236	2,346	0,510	0,520	0,204	0,208
21	12,115	12,225	9,754	9,864	3,902	3,946	23,97	7,140	7,200	6,264	6,324	2,506	2,530	0,525	0,535	0,210	0,214
24	12,950	12,810	10,589	10,449	4,236	4,180	25,71	7,450	7,320	6,574	6,444	2,630	2,578	0,525	0,495	0,210	0,198
43,5	17,670	17,545	15,309	15,184	6,124	6,074	37,26	9,285	9,120	8,409	8,244	3,364	3,298	0,615	0,620	0,246	0,248
46		19,590		17,229		6,892	42,11		10,140		9,264		3,706		0,660		0,264
48	19,670	20,490	17,309	18,129	6,924	7,252	43,30	9,975	10,370	9,099	9,494	3,640	3,798	0,645	0,705	0,258	0,282

maximum yield from 38g DS [g]	hydro correction	
14,4	16,4	Glucose
7,9	8,8	Xylose
1,2	1,4	Arabinose

150-15

duration of test [h]	Glucose		Glucose corrected (without enzyme)		Glucose corrected to 400g mash		Hydrolysed glucane [%]	Xylose		Xylose corrected (without enzyme)		Xylose corrected to 400g mash		Arabinose		Arabinose corrected to 400g mash	
	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2		Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2
2	7,480	7,485	5,119	5,124	2,048	2,050	9,88	6,240	5,640	5,364	4,764	2,146	1,906	0,575	0,600	0,230	0,240
4	6,405	7,860	4,044	5,499	1,618	2,200	9,21	4,935	6,415	4,059	5,539	1,624	2,216	0,445	0,510	0,178	0,204
21	16,110	14,325	13,749	11,964	5,500	4,786	24,81	10,645	10,060	9,769	9,184	3,908	3,674	0,895	0,835	0,358	0,334
26	17,570	15,980	15,209	13,619	6,084	5,448	27,81	11,245	10,850	10,369	9,974	4,148	3,990	0,920	0,795	0,368	0,318
30	18,840	17,280	16,479	14,919	6,592	5,968	30,29	11,515	11,160	10,639	10,284	4,256	4,114	0,970	0,875	0,388	0,350
46,5	21,655	24,170	19,294	21,809	7,718	8,724	39,65	11,870	12,880	10,994	12,004	4,398	4,802	0,715	1,075	0,286	0,430

maximum yield from 38g DS [g]	hydro correction	
18,2	20,7	Glucose
10,0	11,1	Xylose
1,5	1,7	Arabinose

150-20

duration of test [h]	Glucose		Glucose corrected (without enzyme)		Glucose corrected to 400g mash		Hydrolysed glucane [%]	Xylose		Xylose corrected (without enzyme)		Xylose corrected to 400g mash		Arabinose		Arabinose corrected to 400g mash	
	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2		Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2
2	6,740	8,010	4,379	5,649	1,752	2,260	9,67	6,415	7,100	5,539	6,224	2,216	2,490	0,515	0,540	0,206	0,216
4	8,220	9,180	5,859	6,819	2,344	2,728	12,23	6,390	8,130	5,514	7,254	2,206	2,902	0,570	0,565	0,228	0,226
21	12,025	12,905	9,664	10,544	3,866	4,218	19,49	10,205	9,495	9,329	8,619	3,732	3,448	0,680	0,605	0,272	0,242
26	12,745	13,560	10,384	11,199	4,154	4,480	20,82	10,680	9,815	9,804	8,939	3,922	3,576	0,705	0,630	0,282	0,252
30	14,840	11,480	12,479	9,119	4,992	3,648	20,84	11,720	8,070	10,844	7,194	4,338	2,878	0,750	5,150	0,300	2,060
46,5	23,145	18,655	20,784	16,294	8,314	6,518	35,77	12,975	12,355	12,099	11,479	4,840	4,592	0,965	0,780	0,386	0,312

maximum yield from 38g DS [g]	hydro correction	
18,2	20,7	Glucose
10,0	11,1	Xylose
1,5	1,7	Arabinose

150-20

duration of test [h]	Glucose		Glucose corrected (without enzyme)		Glucose corrected to 400g mash		Hydrolysed glucane [%]	Xylose		Xylose corrected (without enzyme)		Xylose corrected to 400g mash		Arabinose		Arabinose corrected to 400g mash	
	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2		Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2
2	7,725	6,955	5,364	4,594	2,146	1,838	9,61	5,515	5,045	4,639	4,169	1,856	1,668	0,580	0,530	0,232	0,212
3	8,510	13,035	6,149	10,674	2,460	4,270	16,23	6,035	6,355	5,159	5,479	2,064	2,192	0,585	0,485	0,234	0,194
22,5	13,500	12,575	11,139	10,214	4,456	4,086	20,60	8,000	8,020	7,124	7,144	2,850	2,858	0,685	0,685	0,274	0,274
24,5	13,270	12,480	10,909	10,119	4,364	4,048	20,29	7,765	7,825	6,889	6,949	2,756	2,780	0,625	0,665	0,250	0,266
27,75	13,105	14,680	10,744	12,319	4,298	4,928	22,25	7,485	8,890	6,609	8,014	2,644	3,206	0,610	0,740	0,244	0,296
48,25	19,145	18,895	16,784	16,534	6,714	6,614	32,14	11,735	9,785	10,859	8,909	4,344	3,564	0,745	0,780	0,298	0,312

maximum yield from 38g DS [g]	hydro correction	
18,2	20,7	Glucose
10,0	11,1	Xylose
1,5	1,7	Arabinose

150-40

duration of test [h]	Glucose		Glucose corrected (without enzyme)		Glucose corrected to 400g mash		Hydrolysed glucane [%]	Xylose		Xylose corrected (without enzyme)		Xylose corrected to 400g mash		Arabinose		Arabinose corrected to 400g mash	
	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2		Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2
18,5	12,790	11,960	10,429	9,599	4,172	3,840	24,47	9,145	8,300	8,269	7,424	3,308	2,970	0,670	0,595	0,268	0,238
21	12,470	12,325	10,109	9,964	4,044	3,986	24,53	8,755	8,300	7,879	7,424	3,152	2,970	0,645	0,580	0,258	0,232
24	13,200	12,990	10,839	10,629	4,336	4,252	26,23	9,000	8,515	8,124	7,639	3,250	3,056	0,625	0,580	0,250	0,232
43,5	17,315	16,650	14,954	14,289	5,982	5,716	35,73	10,500	9,605	9,624	8,729	3,850	3,492	0,705	0,620	0,282	0,248
46	18,535	11,868	16,174	9,507	6,470	3,803	31,38	11,055	10,615	10,179	9,739	4,072	3,896	0,700	0,725	0,280	0,290
48	20,125	19,540	17,764	17,179	7,106	6,872	42,70	11,845	10,955	10,969	10,079	4,388	4,032	0,790	0,740	0,316	0,296

maximum yield from 38g DS [g]	hydro correction	
14,4	16,4	Glucose
7,9	8,8	Xylose
1,2	1,4	Arabinose

150-50

duration of test [h]	Glucose		Glucose corrected (without enzyme)		Glucose corrected to 400g mash		Hydrolysed glucane [%]	Xylose		Xylose corrected (without enzyme)		Xylose corrected to 400g mash		Arabinose		Arabinose corrected to 400g mash	
	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2		Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2
18,5	14,045	14,020	11,684	11,659	4,674	4,664	22,52	9,055	9,375	8,179	8,499	3,272	3,400	0,680	0,670	0,272	0,268
21	14,735	14,050	12,374	11,689	4,950	4,676	23,21	9,240	9,120	8,364	8,244	3,346	3,298	0,680	0,650	0,272	0,260
24	15,270	15,880	12,909	13,519	5,164	5,408	25,50	9,240	10,090	8,364	9,214	3,346	3,686	0,715	0,690	0,286	0,276
43,5	16,035	21,095	13,674	18,734	5,470	7,494	31,26	8,610	11,845	7,734	10,969	3,094	4,388	0,585	0,780	0,234	0,312
46	20,950	23,075	18,589	20,714	7,436	8,286	37,92	11,115	12,750	10,239	11,874	4,096	4,750	0,780	0,815	0,312	0,326
48	21,345	23,560	18,984	21,199	7,594	8,480	38,76	11,290	12,835	10,414	11,959	4,166	4,784	0,770	0,835	0,308	0,334

maximum yield from 38g DS [g]	hydro correction	
18,2	20,7	Glucose
10,0	11,1	Xylose
1,5	1,7	Arabinose

150-50

duration of test [h]	Glucose		Glucose corrected (without enzyme)		Glucose corrected to 400g mash		Hydrolysed glucane [%]	Xylose		Xylose corrected (without enzyme)		Xylose corrected to 400g mash		Arabinose		Arabinose corrected to 400g mash	
	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2		Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2
2	7,460	7,575	5,099	5,214	2,040	2,086	9,95	5,580	6,020	4,704	5,144	1,882	2,058	0,420	0,515	0,168	0,206
4	8,140	7,470	5,779	5,109	2,312	2,044	10,50	6,570	7,255	5,694	6,379	2,278	2,552	0,605	0,530	0,242	0,212
21	14,705	14,410	12,344	12,049	4,938	4,820	23,53	9,225	9,435	8,349	8,559	3,340	3,424	0,605	0,645	0,242	0,258
26	14,900	14,980	12,539	12,619	5,016	5,048	24,27	9,050	9,655	8,174	8,779	3,270	3,512	0,605	0,635	0,242	0,254
30	17,640	15,525	15,279	13,164	6,112	5,266	27,44	10,425	9,815	9,549	8,939	3,820	3,576	0,670	0,630	0,268	0,252
46,5	23,750	18,350	21,389	15,989	8,556	6,396	36,06	13,270	10,210	12,394	9,334	4,958	3,734	1,065	0,835	0,426	0,334

maximum yield from 38g DS [g]	hydro correction	
18,2	20,7	Glucose
10,0	11,1	Xylose
1,5	1,7	Arabinose

155-40

duration of test [h]	Glucose		Glucose corrected (without enzyme)		Glucose corrected to 400g mash		Hydrolysed glucane [%]	Xylose		Xylose corrected (without enzyme)		Xylose corrected to 400g mash		Arabinose		Arabinose corrected to 400g mash	
	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2		Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2
2	1,139	1,392	3,334	4,599	-	-	14,95	1,063	1,033	4,439	4,289	-	-	0,118	0,108	0,590	0,540
4	1,551	1,666	5,394	5,969	-	-	22,23	1,165	1,141	4,949	4,829	-	-	0,117	0,119	0,585	0,595
22	2,942	2,812	12,349	11,699	-	-	48,28	1,509	1,418	6,669	6,214	-	-	0,136	0,127	0,680	0,635
24	2,975	2,931	12,514	12,294	-	-	49,51	1,502	1,442	6,634	6,334	-	-	0,130	0,127	0,650	0,635
26	3,338	3,144	14,329	13,359	-	-	55,73	1,644	1,518	7,344	6,714	-	-	0,144	0,140	0,720	0,700
28	3,496	3,261	15,119	13,944	-	-	58,61	1,708	1,535	7,664	6,799	-	-	0,156	0,141	0,780	0,705
30	3,421	3,137	14,744	13,324	-	-	56,79	1,631	1,469	7,279	6,469	-	-	0,147	0,128	0,735	0,640
48	4,524	3,677	20,259	16,024	-	-	75,00	1,908	1,561	8,664	6,929	-	-	0,168	0,138	0,840	0,690
50	4,694	3,762	21,109	16,449	-	-	77,82	1,942	1,592	8,834	7,084	-	-	0,168	0,144	0,840	0,720
52	4,869	4,043	21,984	17,854	-	-	82,01	2,006	1,706	9,154	7,654	-	-	0,171	0,157	0,855	0,785
54	4,896	3,939	22,119	17,334	-	-	81,67	1,988	1,640	9,064	7,324	-	-	0,166	0,144	0,830	0,720
72	6,321	4,952	29,244	22,399	-	-	107,29	2,378	1,920	11,014	8,724	-	-	0,195	0,170	0,975	0,850
74	6,398	5,082	29,629	23,049	-	-	109,18	2,392	1,953	11,084	8,889	-	-	0,200	0,170	1,000	0,850
77	5,232	4,700	23,799	21,139	-	-	91,18	1,941	1,787	8,829	8,059	-	-	0,164	0,153	0,820	0,765

maximum yield from 38g DS [g]	hydro correction	
18,2	20,7	Glucose
10,0	11,1	Xylose
1,5	1,7	Arabinose

160-60

duration of test [h]	Glucose		Glucose corrected (without enzyme)		Glucose corrected to 400g mash		Hydrolysed glucane [%]	Xylose		Xylose corrected (without enzyme)		Xylose corrected to 400g mash		Arabinose		Arabinose corrected to 400g mash	
	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2		Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2
2	1,946	1,948	-	-	6,632	6,641	2,64	0,855	0,983	-	-	2,991	3,554	0,108	0,109	0,475	0,480
4	2,319	2,583	-	-	8,311	9,499	2,91	0,943	1,097	-	-	3,378	4,056	0,102	0,114	0,449	0,502
24	4,756	4,863	-	-	19,277	19,759	4,53	1,465	1,561	-	-	5,675	6,098	0,155	0,145	0,682	0,638
26	5,086	4,974	-	-	20,762	20,258	4,63	1,498	1,565	-	-	5,820	6,115	0,141	0,153	0,620	0,673
28	5,259	5,170	-	-	21,541	21,140	4,68	1,513	1,584	-	-	5,886	6,199	0,144	0,148	0,634	0,651
50	6,412	6,291	-	-	26,729	26,185	5,47	1,768	1,778	-	-	7,008	7,052	0,158	0,168	0,695	0,739
52	6,211	6,079	-	-	25,825	25,231	5,24	1,696	1,721	-	-	6,692	6,802	0,147	0,167	0,647	0,735
54	6,706	6,444	-	-	28,052	26,873	5,61	1,815	1,800	-	-	7,215	7,149	0,161	0,179	0,708	0,788
72	7,729	7,857	-	-	32,656	33,232	6,28	2,031	2,105	-	-	8,166	8,491	0,188	0,193	0,827	0,849

160-70

duration of test [h]	Glucose		Glucose corrected (without		Glucose corrected to 400g		Hydrolysed glucane [%]	Xylose		Xylose corrected (without		Xylose corrected to 400g		Arabinose		Arabinose corrected to	
	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2		Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2
42	3,266	3,620	-	-	14,591	16,361	41,018	1,003	1,128	-	-	4,370	4,995	0,084	0,095	0,420	0,475
48	4,059	3,611	-	-	18,556	16,316	46,213	1,210	1,098	-	-	5,405	4,845	0,108	0,085	0,540	0,425
72,5	4,076	5,165	-	-	18,641	24,086	56,622	1,125	1,421	-	-	4,980	6,460	0,089	0,109	0,445	0,545
74	5,237	4,321	-	-	24,446	19,866	58,723	1,445	1,191	-	-	6,580	5,310	0,108	0,096	0,540	0,480
96	5,790	4,823	-	-	27,211	22,376	65,713	1,571	1,295	-		7,210	5,830	0,129	0,095	0,645	0,475

160-80

duration of test [h]	Glucose		Glucose corrected (without		Glucose corrected to 400g		Hydrolysed glucane [%]	Xylose		Xylose corrected (without		Xylose corrected to 400g		Arabinose		Arabinose corrected to	
	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2		Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2
2	1,598	1,647	-	-	5,505	5,750	10,518	0,807	0,825	-	-	3,113	3,203	0,054	0,052	0,270	0,260
4	1,854	1,886	-	-	6,785	6,945	12,831	0,892	0,885	-	-	3,538	3,503	0,054	0,068	0,270	0,340
21	2,627	2,614	-	-	10,650	10,585	19,845	1,027	0,995	-	-	4,213	4,053	0,074	0,071	0,370	0,355
22	2,979	3,199	-	-	12,410	13,510	24,224	1,138	1,195	-	-	4,768	5,053	0,057	0,081	0,285	0,405
24	3,332	3,142	-	-	14,175	13,225	25,607	1,460	1,157	-	-	6,378	4,863	0,077	0,065	0,385	0,325
27	3,194	3,444	-	-	13,485	14,735	26,373	1,180	1,240	-	-	4,978	5,278	0,068	0,070	0,340	0,350
29	3,771	4,185	-	-	16,370	18,440	32,532	1,280	1,371	-	-	5,478	5,933	0,081	0,089	0,405	0,445
45	4,244	4,690	-	-	18,735	20,965	37,102	1,433	1,507	-	-	6,243	6,613	0,093	0,107	0,465	0,535
48	4,124	4,775	-	-	18,135	21,390	36,939	1,379	1,560	-	-	5,973	6,878	0,092	0,105	0,460	0,525
50	4,459	4,752	-	-	19,810	21,275	38,397	1,462	1,517	-	-	6,388	6,663	0,091	0,099	0,455	0,495
72	4,297	5,816	-	-	19,000	26,595	42,612	1,318	1,699	-	-	5,668	7,573	0,090	0,130	0,450	0,650
74	5,480	6,312	-	-	24,915	29,075	50,457	1,656	1,847	-	-	7,358	8,313	0,121	0,133	0,605	0,665
76	5,426	6,421	-	-	24,645	29,620	50,714	1,624	1,875	-	-	7,198	8,453	0,108	0,134	0,540	0,670
78	5,481	6,275	-	-	24,920	28,890	50,289	1,643	1,833	-	-	7,293	8,243	0,118	0,111	0,590	0,555
96	6,710	8,024	-	-	31,065	37,635	64,205	1,908	2,225	-	-	8,618	10,203	0,131	0,169	0,655	0,845

160-90

duration of test [h]	Glucose		Glucose corrected (without		Glucose corrected to 400g		Hydrolysed glucane [%]	Xylose		Xylose corrected (without		Xylose corrected to 400g		Arabinose		Arabinose corrected to	
	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2		Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2	Versuch 1	Versuch 2
3,75	0,357	1,099	-	-	1,702	3,685	8,5	0,632	-	-	-	1,340	-	0,429	-	2,129	-
3,75	0,361	1,099	-	-	1,721	3,685	8,5	0,612	-	-	-	1,242	-	0,399	-	1,980	-
17	0,334	2,052	-	-	1,589	8,369	19,4	0,817	-	-	-	2,250	-	0,438	-	2,173	-
17	0,370	2,238	-	-	1,766	9,283	21,5	0,887	-	-	-	2,594	-	0,441	-	2,188	-
22	0,319	2,385	-	-	1,515	10,005	23,2	0,886	-	-	-	2,589	-	0,478	-	2,372	-
22	0,387	2,716	-	-	1,849	11,632	27,0	1,004	-	-	-	3,169	-	0,505	-	2,506	-
48	0,357	3,193	-	-	1,702	13,976	32,4	1,036	-	-	-	3,326	-	0,514	-	2,550	-
48	0,468	3,703	-	-	2,247	16,482	38,2	1,197	-	-	-	4,118	-	0,538	-	2,669	-
51	0,372	3,373	-	-	1,775	14,860	34,5	1,087	-	-	-	3,577	-	0,534	-	2,649	-
51	0,491	3,987	-	-	2,360	17,877	41,5	1,266	-	-	-	4,457	-	0,574	-	2,848	-
72	0,398	3,904	-	-	1,903	17,470	40,5	1,162	-	-	-	3,946	-	0,534	-	2,649	-
72	0,53	4,595	-	-	2,552	20,865	48,4	1,345	-	-	-	4,845	-	0,557	-	2,764	-
96	0,451	4,619	-	-	2,164	20,983	48,7	1,309	-	-	-	4,668	-	0,588	-	2,917	-
96	0,617	5,558	-	-	2,979	25,598	59,4	1,543	-	-	-	5,818	-	0,622	-	3,086	-

2. The influence of dispersing the raw material before the direct heated pre-treatment on its enzymatic digestibility

160-30 6,5%DS dispersed maize silage

duration of hydrolysis [h]	Cellobiose		Glucose			Xyl/Gal/Man		Arabinose	
	HPLC [g/l]	corrected	HPLC [g/l]	corrected	yield	HPLC [g/l]	corrected	HPLC [g/l]	corrected
6	2,610	2,567	8,057	6,663	18,91	3,779	2,344	3,943	3,943
16	4,036	3,993	20,137	18,743	53,20	5,347	3,912	4,320	4,320
24	4,027	3,984	21,142	19,748	56,06	4,808	3,373	3,750	3,750
41	3,824	3,781	24,187	22,793	64,70	4,783	3,348	4,368	4,368
48	3,774	3,731	25,002	23,608	67,01	4,950	3,515	4,484	4,484
65	3,630	3,587	28,152	26,758	75,95	5,159	3,724	4,483	4,483
72	3,325	3,282	27,301	25,907	73,54	5,183	3,748	4,209	4,209
144	2,970	2,927	26,410	25,016	71,01	5,100	3,665	4,283	4,283

160-30 6,5%DS

duration of hydrolysis [h]	Cellobiose		Glucose			Xyl/Gal/Man		Arabinose	
	HPLC [g/l]	corrected	HPLC [g/l]	corrected	yield	HPLC [g/l]	corrected	HPLC [g/l]	corrected
48	4,251	4,208	24,157	22,763	64,61	5,060	3,625	4,129	4,129
48	3,669	3,626	17,694	16,300	46,27	4,362	2,927	3,304	3,304

160-50 5,27%DS dispersed maize silage

duration of hydrolysis [h]	Cellobiose		Glucose			Xyl/Gal/Man		Arabinose	
	HPLC [g/l]	corrected	HPLC [g/l]	corrected	yield	HPLC [g/l]	corrected	HPLC [g/l]	corrected
18	3,284	3,249	12,958	11,828	41,41	3,940	2,777	2,655	2,655
24	3,793	3,758	14,925	13,795	48,30	4,256	3,093	2,814	2,814
25	3,124	3,089	14,687	13,557	47,46	3,882	2,719	2,866	2,866
48	3,842	3,807	18,553	17,423	61,00	4,174	3,011	2,880	2,880
49	4,057	4,022	19,286	18,156	63,56	4,431	3,268	2,681	2,681
65	3,759	3,724	20,391	19,261	67,43	4,449	3,286	2,744	2,744
94,5	3,330	3,295	21,662	20,532	71,88	4,330	3,167	3,010	3,010
116	3,249	3,214	22,488	21,358	74,77	4,417	3,254	3,035	3,035
118	3,323	3,288	21,558	20,428	71,52	4,477	3,314	2,751	2,751
139	3,142	3,107	22,448	21,318	74,63	4,440	3,277	3,008	3,008
144	3,092	3,057	22,794	21,664	75,85	4,555	3,392	2,806	2,806
144	3,090	3,055	22,179	21,049	73,69	4,511	3,348	2,722	2,722

160-50 5,27%DS

duration of hydrolysis [h]	Cellobiose		Glucose			Xyl/Gal/Man		Arabinose	
	HPLC [g/l]	corrected	HPLC [g/l]	corrected	yield	HPLC [g/l]	corrected	HPLC [g/l]	corrected
48	3,314	3,279	22,832	21,702	75,98	4,710	3,547	3,154	3,154
48	3,602	3,567	23,832	23,968	83,91	4,844	3,681	4,318	4,318

160-50 7,25%DS

duration of hydrolysis [h]	Cellobiose		Glucose			Xyl/Gal/Man		Arabinose	
	HPLC [g/l]	corrected	HPLC [g/l]	corrected	yield	HPLC [g/l]	corrected	HPLC [g/l]	corrected
3	0,891	4,406	0,936	3,072	7,60	0,600	1,344	0,510	2,550
3	0,976	4,831	0,910	2,942	7,28	0,640	1,544	0,498	2,490
8	0,759	3,746	1,344	5,112	12,65	0,796	2,324	0,547	2,735
8	0,752	3,711	1,392	5,352	13,24	0,786	2,274	0,547	2,735
23	0,481	2,356	2,421	10,497	25,97	0,981	3,249	0,594	2,970
23	0,491	2,406	2,389	10,337	25,57	0,973	3,209	0,598	2,990
29	0,448	2,191	2,713	11,957	29,58	1,027	3,479	0,612	3,060
29	0,472	2,311	2,738	12,082	29,89	1,050	3,594	0,630	3,150
47	0,441	2,156	3,358	15,182	37,56	1,121	3,949	0,584	2,920
47	0,471	2,306	3,457	15,677	38,78	1,157	4,129	0,651	3,255
72	0,451	2,206	4,323	20,007	49,49	1,316	4,924	0,671	3,355
72	0,482	2,361	4,458	20,682	51,16	1,369	5,189	0,728	3,640
79	0,499	2,446	4,719	21,987	54,39	1,407	5,379	0,733	3,665
79	0,516	2,531	4,773	22,257	55,06	1,440	5,544	0,773	3,865
96	0,603	2,966	5,785	27,317	67,58	1,651	6,599	0,828	4,140
96	0,569	2,796	5,546	26,122	64,62	1,608	6,384	0,813	4,065

3. The influence of the pre-treatment conditions on the enzymatic hydrolysis of dispersed corn silage using indirect heating for pre-treatment

135-30 16,67%DS									
duration of hydrolysis [h]	Cellobiose		Glucose			Xyl/Gal/Man		Arabinose	
	HPLC [g/l]	corrected	HPLC [g/l]	corrected	yield	HPLC [g/l]	corrected	HPLC [g/l]	corrected
24	6,627	6,517	28,718	25,144	27,83	5,780	2,100	6,021	6,021
24	8,254	8,144	33,777	30,203	33,43	6,365	2,685	6,592	6,592
48	4,033	3,923	36,151	32,577	36,06	6,439	2,759	6,253	6,253
48	4,295	4,185	38,343	34,769	38,48	7,028	3,348	6,368	6,368
69	4,634	4,524	40,554	36,980	40,93	7,196	3,516	6,257	6,257
69	4,163	4,053	40,024	36,450	40,34	8,225	4,545	6,348	6,348

140-90 19,97%DS									
duration of hydrolysis [h]	Cellobiose		Glucose			Xyl/Gal/Man		Arabinose	
	HPLC [g/l]	corrected	HPLC [g/l]	corrected	yield	HPLC [g/l]	corrected	HPLC [g/l]	corrected
0	0,460	-	2,200	2,200	2,03	0,940	0,940	2,640	2,640
24	5,407	5,276	39,439	35,158	32,48	14,550	10,142	5,723	5,723
24	5,867	5,736	40,898	36,617	33,83	14,017	9,609	7,466	7,466
48	6,982	6,850	48,263	43,981	40,64	18,466	14,057	7,124	7,124
48	8,772	8,641	53,792	49,510	45,75	19,742	15,334	7,040	7,040
70,5	5,015	4,884	47,036	42,754	39,50	11,923	7,515	7,239	7,239
70,5	5,672	5,541	50,844	46,562	43,02	12,939	8,531	7,821	7,821

145-30 12,51%DS									
duration of hydrolysis [h]	Cellobiose		Glucose			Xyl/Gal/Man		Arabinose	
	HPLC [g/l]	corrected	HPLC [g/l]	corrected	yield	HPLC [g/l]	corrected	HPLC [g/l]	corrected
0		0,000	1,050	1,050	1,55	0,440	0,440	1,050	1,050
24	1,805	1,723	28,668	25,986	38,34	6,764	4,003	5,419	5,419
24	2,100	2,018	30,570	27,888	41,14	7,894	5,133	5,623	5,623
48	1,861	1,779	34,179	31,497	46,47	8,176	5,415	5,441	5,441
48	1,862	1,780	32,833	30,151	44,48	7,454	4,693	5,552	5,552
69	1,819	1,737	32,526	29,844	44,03	6,947	4,186	5,647	5,647
69	1,976	1,894	33,991	31,309	46,19	7,597	4,836	5,650	5,650

145-90 16,22%DS									
duration of hydrolysis [h]	Cellobiose		Glucose			Xyl/Gal/Man		Arabinose	
	HPLC [g/l]	corrected	HPLC [g/l]	corrected	yield	HPLC [g/l]	corrected	HPLC [g/l]	corrected
0	0,650	-	1,970	1,970	2,24	1,190	1,190	2,800	2,800
24	6,386	6,255	40,677	36,402	41,40	13,838	9,436	7,865	7,865
24	-	-	40,370	36,095	41,05	15,061	10,659	7,445	7,445
48	7,331	7,200	50,945	46,670	53,08	16,779	12,377	7,268	7,268
48	7,216	7,085	47,368	43,093	49,01	15,468	11,066	6,799	6,799
70,5	5,413	5,282	49,016	44,741	50,88	15,719	11,317	8,566	8,566
70,5	7,098	6,967	48,651	44,376	50,47	16,624	12,222	7,817	7,817

150-30 10,62%DS									
duration of hydrolysis [h]	Cellobiose		Glucose			Xyl/Gal/Man		Arabinose	
	HPLC [g/l]	corrected	HPLC [g/l]	corrected	yield	HPLC [g/l]	corrected	HPLC [g/l]	corrected
0		0,000	1,934	1,934	3,36	2,073	2,073	1,849	1,849
24	3,366	3,296	21,129	18,851	32,74	8,213	5,868	5,920	5,920
24	3,311	3,241	20,989	18,711	32,50	7,968	5,623	5,976	5,976
48	3,170	3,100	23,624	21,346	37,08	8,996	6,651	5,571	5,571
48	2,969	2,899	22,946	20,668	35,90	8,484	6,139	5,789	5,789
72	3,211	3,141	27,151	24,873	43,20	8,948	6,603	5,921	5,921
72	2,737	2,667	24,983	22,705	39,44	7,865	5,520	5,910	5,910

150-30 14,09%DS									
duration of hydrolysis [h]	Cellobiose		Glucose			Xyl/Gal/Man		Arabinose	
	HPLC [g/l]	corrected	HPLC [g/l]	corrected	yield	HPLC [g/l]	corrected	HPLC [g/l]	corrected
0	0,210		0,410	0,410	0,56	0,3600	0,3600	1,1100	1,1100
24	7,770	7,677	29,735	26,714	36,38	5,3520	2,2413	5,4580	5,4580
24	8,254	8,161	33,360	30,339	41,31	5,4280	2,3173	5,8690	5,8690
48	6,557	6,464	38,342	35,321	51,24	6,1790	3,0683	5,6600	5,6600
48	7,637	7,544	42,978	39,957	52,32	6,9580	3,8473	6,0650	6,0650
69	6,689	6,596	39,036	36,015	47,15	6,2460	3,1353	5,3760	5,3760
69	6,743	6,650	40,733	37,712	49,38	6,1410	3,0303	5,8840	5,8840

150-60 15,52%DS									
duration of hydrolysis [h]	Cellobiose		Glucose			Xyl/Gal/Man		Arabinose	
	HPLC [g/l]	corrected	HPLC [g/l]	corrected	yield	HPLC [g/l]	corrected	HPLC [g/l]	corrected
24	4,013	3,911	29,320	25,992	30,89	6,822	3,395	5,882	5,882
24	3,296	3,194	24,063	20,735	24,64	6,350	2,923	4,993	4,993
48	5,390	5,288	40,787	37,459	44,52	9,382	5,955	7,473	7,473
48	5,462	5,360	41,679	38,351	45,58	10,123	6,696	7,177	7,177
71	4,647	4,545	42,942	39,614	47,08	8,568	5,141	8,640	8,640
71	4,510	4,408	41,030	37,702	44,81	10,517	7,090	5,888	5,888

150-70 20,23%DS									
duration of hydrolysis [h]	Cellobiose		Glucose			Xyl/Gal/Man		Arabinose	
	HPLC [g/l]	corrected	HPLC [g/l]	corrected	yield	HPLC [g/l]	corrected	HPLC [g/l]	corrected
0	0,690		1,150	1,150	1,09	0,960	0,960	2,280	2,280
24	32,754	32,621	50,432	46,095	43,73	17,714	13,249	8,233	8,233
24	31,814	31,681	47,542	43,205	40,99	16,353	11,888	7,565	7,565
48	26,385	26,252	60,239	55,902	56,50	19,515	15,050	8,798	8,798
48	27,554	27,421	65,818	61,481	56,08	19,854	15,389	7,723	7,723
69	24,873	24,740	71,709	67,372	61,46	18,740	14,275	8,792	8,792
69	23,771	23,638	67,414	63,077	57,54	18,989	14,524	7,151	7,151

150-90 20,31%DS									
duration of hydrolysis [h]	Cellobiose		Glucose			Xyl/Gal/Man		Arabinose	
	HPLC [g/l]	corrected	HPLC [g/l]	corrected	yield	HPLC [g/l]	corrected	HPLC [g/l]	corrected
0	0,740		1,550	1,550	1,41	1,280	1,280	2,780	2,780
24	3,515	3,382	27,692	23,337	21,20	6,592	2,109	5,583	5,583
24	3,878	3,745	28,183	23,828	21,65	6,817	2,334	5,488	5,488
48	7,467	7,334	52,448	48,093	43,69	19,708	15,225	7,150	7,150
48	6,630	6,497	48,848	44,493	40,42	17,482	12,999	7,017	7,017

150-120 16,10%DS

duration of hydrolysis [h]	Cellobiose		Glucose			Xyl/Gal/Man		Arabinose	
	HPLC [g/l]	corrected	HPLC [g/l]	corrected	yield	HPLC [g/l]	corrected	HPLC [g/l]	corrected
0		0,800		2,200	1,86		1,100		0,760
24	6,517	6,411	20,525	17,074	19,57	18,415	14,861	8,548	8,548
24	6,167	6,061	20,250	16,798	19,25	18,467	14,913	8,014	8,014
48	6,646	6,540	24,687	21,235	24,34	20,513	16,959	8,046	8,046
48	7,116	7,010	24,334	20,882	23,93	20,121	16,567	9,036	9,036

155-30 15,13%DS

duration of hydrolysis [h]	Cellobiose		Glucose			Xyl/Gal/Man		Arabinose	
	HPLC [g/l]	corrected	HPLC [g/l]	corrected	yield	HPLC [g/l]	corrected	HPLC [g/l]	corrected
0		0,000	1,570	1,570	1,91	0,600	0,600	1,790	1,790
24	7,508	7,409	40,929	37,685	45,95	16,780	13,440	8,830	8,830
24	7,329	7,230	38,565	35,321	43,07	15,772	12,432	7,077	7,077
48	3,417	3,318	34,417	31,173	38,01	7,377	4,037	5,904	5,904
48	3,331	3,232	33,407	30,163	36,78	7,814	4,474	5,344	5,344
48	3,638	3,539	34,474	31,230	38,08	7,829	4,489	5,865	5,865
48	4,077	3,978	35,502	32,258	39,34	8,542	5,202	5,948	5,948
72	4,490	4,391	38,771	35,527	43,32	8,556	5,216	6,033	6,033
72	3,382	3,283	36,966	33,722	41,12	8,122	4,782	5,972	5,972

155-90 18,12%DS

duration of hydrolysis [h]	Cellobiose		Glucose			Xyl/Gal/Man		Arabinose	
	HPLC [g/l]	corrected	HPLC [g/l]	corrected	yield	HPLC [g/l]	corrected	HPLC [g/l]	corrected
0	1,07	0,80	3,23	2,20	1,86	1,580	1,100	2,840	0,760
24	8,69	8,57	44,36	40,48	41,21	18,217	14,217	7,439	7,439
24	8,88	8,76	43,89	40,00	40,73	18,059	14,059	7,840	7,840
48	11,61	11,49	60,28	56,40	57,42	21,161	17,161	6,844	6,844
48	9,91	9,79	58,96	55,08	56,08	19,927	15,927	7,732	7,732
72	10,00	9,88	63,23	59,34	60,42	21,449	17,449	7,006	7,006
72	9,65	9,53	63,47	59,59	60,67	21,485	17,485	6,633	6,633

4. The Optimization of the enzyme dosage using GC 220 for enzymatic hydrolysis

180µl GC 220/gDS							
hydrolysis duration [h]	Glucose [g/l]	Glucose corrected [g/l]	Glucose yield [%]	Xylose [g/l]	Xylose corrected [g/l]	Arabinose [g/l]	Arabinose corrected [g/l]
19	6,740	20,077	30,41	1,414	4,147	1,817	3,111
26	7,246	21,595	32,71	1,580	4,645	1,896	3,348
45	7,699	22,954	34,76	1,714	5,047	1,859	3,237
116	26,789	26,646	40,36	5,931	5,836	5,433	3,093

200µl GC 220/gDS							
hydrolysis duration [h]	Glucose [g/l]	Glucose corrected [g/l]	Glucose yield [%]	Xylose [g/l]	Xylose corrected [g/l]	Arabinose [g/l]	Arabinose corrected [g/l]
19	6,500	19,342	29,29	1,417	4,146	1,773	2,719
26	7,195	21,427	32,45	1,583	4,644	1,844	2,932
45	7,818	23,296	35,28	1,692	4,971	1,907	3,121
116	26,289	26,146	39,60	5,887	5,792	5,523	3,183

250µl GC 220/gDS							
hydrolysis duration [h]	Glucose [g/l]	Glucose corrected [g/l]	Glucose yield [%]	Xylose [g/l]	Xylose corrected [g/l]	Arabinose [g/l]	Arabinose corrected [g/l]
19	6,945	20,637	31,26	1,614	4,710	1,988	2,714
26	7,379	21,939	33,23	1,732	5,064	1,991	2,723
45	7,626	22,680	34,35	1,767	5,169	1,983	2,699
116	27,245	27,102	41,05	6,263	6,168	6,425	4,085

300µl GC 220/gDS							
hydrolysis duration [h]	Glucose [g/l]	Glucose corrected [g/l]	Glucose yield [%]	Xylose [g/l]	Xylose corrected [g/l]	Arabinose [g/l]	Arabinose corrected [g/l]
19	7,555	22,427	33,97	1,675	4,867	2,218	2,754
26	8,098	24,056	36,43	1,802	5,248	2,265	2,895
45	8,159	24,239	36,71	1,821	5,305	2,262	2,886
116	29,287	29,144	44,14	6,361	6,266	7,508	5,168

180µl GC 880/gDS							
hydrolysis duration [h]	Glucose [g/l]	Glucose corrected [g/l]	Glucose yield [%]	Xylose [g/l]	Xylose corrected [g/l]	Arabinose [g/l]	Arabinose corrected [g/l]
24	26,692	23,363	27,77	6,586	3,159	5,438	5,438
48	41,233	37,905	45,05	9,753	6,326	7,325	7,325
71	41,986	38,658	45,95	9,543	6,116	7,264	7,264

5. The influence of different pre-hydrolysis times on the SSF process

Test	Duration SSF [h]	Total hydrolysis time [h]	Ethanol yield [%]	hydrolysed cellulose [%]	hydrolysed glucane [%]
160-30 21h prehydrolysis before SSF	4,0	25,0	0,67	-	28,59
	3,0	24,0	0,71	-	26,27
	21,0	42,0	2,32	-	34,49
	22,0	43,0	3,13	-	33,36
	45,0	66,0	7,42	-	39,50
	51,0	72,0	9,45	-	39,71
	70,0	91,0	47,55	-	51,23
	73,0	94,0	63,10	-	64,57
	94,0	115,0	52,70	-	55,17
	118,0	139,0	72,06	16,95	73,30
	121,0	142,0	80,59	55,16	82,03
	141,0	162,0	78,25	44,01	79,48
	142,0	163,0	73,88	26,01	75,37
	150,0	171,0	84,70	72,33	85,96
	165,0	186,0	85,43	77,71	87,19
	166,0	187,0	83,65	71,40	85,75
150-50 4h prehydrolysis before SSF	16,5	20,5	5,14	-	25,69
	37,5	41,5	29,95	-	34,55
	44,0	48,0	38,90	-	40,26
	62,0	66,0	47,57	-	49,10
	67,0	71,0	49,40	-	50,78
	86,0	90,0	56,80	-	58,41
	158,0	162,0	79,26	40,62	80,48
150-50 23h prehydrolysis before SSF	18,5	41,5	4,22	-	30,06
	25,0	48,0	8,05	-	33,57
	43,0	66,0	41,29	-	43,03
	46,0	69,0	43,70	-	45,07
	67,0	90,0	54,74	-	56,24
	139,0	162,0	81,22	49,66	82,64
150-50 24h prehydrolysis before SSF	17,5	41,5	10,49	-	30,34
	24,0	48,0	18,93	-	33,51
	42,0	66,0	40,14	-	41,89
	45,0	69,0	42,94	-	44,34
	66,0	90,0	52,16	-	53,39
	138,0	162,0	77,42	32,17	78,51

160-30 21h GC 220 plus Triticale SSF

Duration SSF [h]	Glucose										Xyl/Gal/Man								Arabinose							
	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected	mean [g/l]	Standard deviation	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected
3	29,474	58,948	28,015	56,03	27,78	55,56	29,015	58,03	57,14	1,61	5,448	10,896	5,117	10,234	5,526	11,052	5,304	10,608	3,098	9,294	3,062	9,186	2,96	8,88	3,025	9,075
4	32,047	64,094	30,142	60,284	30,174	60,348	32,473	64,946	62,42	2,45	6,004	12,008	5,938	11,876	5,949	11,898	5,951	11,902	3,179	9,537	3,219	9,657	3,244	9,732	3,089	9,267
21	33,797	67,594	35,543	71,086	36,767	73,534	37,713	75,426	71,91	3,38	6,372	12,744	6,213	12,426	6,438	12,876	6,638	13,276	3,056	9,168	3,146	9,438	3,146	9,438	3,198	9,594
22	35,6	71,2	33,912	67,824	33,048	66,096	32,588	65,176	67,57	2,65	6,068	12,136	5,583	11,166	6,077	12,154	5,73	11,46	3,182	9,546	3,112	9,336	2,987	8,961	3,004	9,012
45	33,549	67,098	35,828	71,656	35,586	71,172	38,485	76,97	71,72	4,05	6,517	13,034	6,498	12,996	6,38	12,76	6,817	13,634	2,948	8,844	3,109	9,327	2,983	8,949	3,077	9,231
51	32,02	64,04	35,338	70,676	32,26	64,52	35,702	71,404	67,66	3,92	6,175	12,35	6,436	12,872	6,019	12,038	6,401	12,802	3,057	9,171	3,039	9,117	2,956	8,868	3,074	9,222
70	2,159	4,318	9,248	18,496	2,391	4,782	2,652	5,304	8,23	6,86	3,357	6,714	4,548	9,096	3,324	6,648	3,482	6,964	2,712	8,136	3,067	9,201	2,72	8,16	2,779	8,337
73	1,593	3,186	1,522	3,044	1,793	3,586	1,682	3,364	3,30	0,23	2,834	5,668	2,597	5,194	3,11	6,22	2,796	5,592	2,659	7,977	2,56	7,68	2,65	7,95	2,605	7,815
94	4,668	9,336	2,01	4,02	2,322	4,644	2,064	4,128	5,53	2,55	3,319	6,638	2,709	5,418	3,238	6,476	2,857	5,714	2,611	7,833	2,582	7,746	2,725	8,175	2,67	8,01
118	1,273	2,546	1,226	2,452	1,593	3,186	1,438	2,876	2,77	0,33	2,831	5,662	2,542	5,084	3,024	6,048	2,826	5,652	2,604	7,812	2,5	7,5	2,687	8,061	2,61	7,83
121	1,316	2,632	1,461	2,922	1,704	3,408	1,987	3,974	3,23	0,59	3,31	6,62	3,174	6,348	3,187	6,374	3,398	6,796	2,782	8,346	2,735	8,205	2,746	8,238	2,915	8,745
141	1,209	2,418	1,169	2,338	1,613	3,226	1,542	3,084	2,77	0,45	2,871	5,742	2,636	5,272	3,104	6,208	2,715	5,43	2,678	8,034	2,541	7,623	2,719	8,157	2,563	7,689
142	1,429	2,858	1,603	3,206	1,743	3,486	1,888	3,776	3,33	0,39	3,323	6,646	3,302	6,604	3,187	6,374	3,277	6,554	2,807	8,421	2,789	8,367	2,729	8,187	2,846	8,538
150	1,036	2,072	1,143	2,286	1,612	3,224	1,849	3,698	2,82	0,77	3,181	6,362	2,938	5,876	3,509	7,018	3,152	6,304	2,387	7,161	2,35	7,05	2,493	7,479	2,47	7,41
165	1,206	2,412	1,319	2,638	2,349	4,698	2,975	5,95	3,92	1,70	3,174	6,348	2,906	5,812	3,406	6,812	3,051	6,102	2,39	7,17	2,317	6,951	2,469	7,407	2,436	7,308
166	1,374	2,748	1,912	3,824	2,668	5,336	3,436	6,872	4,70	1,80	3,139	6,278	3,152	6,304	3,195	6,39	3,387	6,774	2,66	7,98	2,787	8,361	2,761	8,283	2,831	8,493

Duration SSF [h]	Ethanol													
	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected	mean [g/l]	Standard deviation	Yield [%]	Ethanol yield from Cellulose [%]	total hydrolysed Glucan [%]	hydrolysed Cellulose [%]
3	0,327	0,892	0,264	0,720	0,293	0,799	0,304	0,829	0,81	0,07	0,71	-334,25	26,27	-222,461
4	0,489	1,334	0,227	0,619	0,166	0,453	0,238	0,649	0,76	0,39	0,67	-334,43	28,59	-212,316
21	1,514	4,129	0,764	2,084	0,846	2,307	0,759	2,070	2,65	0,99	2,32	-327,20	34,49	-186,520
22	1,186	3,235	1,255	3,423	1,333	3,635	1,462	3,987	3,57	0,32	3,13	-323,67	33,36	-191,464
45	3,329	9,079	2,839	7,743	3,150	8,591	3,084	8,411	8,46	0,55	7,42	-304,92	39,50	-164,602
51	4,236	11,553	3,173	8,654	4,381	11,948	4,007	10,928	10,77	1,47	9,45	-296,04	39,71	-163,673
70	20,097	54,810	16,071	43,830	21,418	58,413	21,922	59,787	54,21	7,23	47,55	-129,41	51,23	-113,315
73	26,232	71,542	26,258	71,613	25,721	70,148	27,298	74,449	71,94	1,80	63,10	-61,40	64,57	-54,955
94	20,479	55,852	22,399	61,088	22,015	60,041	23,229	63,352	60,08	3,14	52,70	-106,88	55,17	-96,054
118	30,485	83,141	29,657	80,883	29,159	79,525	31,197	85,083	82,16	2,46	72,06	-22,20	73,30	-16,788
121	32,396	88,353	31,958	87,158	34,475	94,023	35,929	97,988	91,88	5,05	80,59	15,10	82,03	21,427
141	33,218	90,595	32,467	88,546	31,298	85,358	33,859	92,343	89,21	3,00	78,25	4,86	79,48	10,270
142	30,056	81,971	29,763	81,172	32,057	87,428	31,662	86,351	84,23	3,12	73,88	-14,25	75,37	-7,728
150	35,326	96,344	35,298	96,267	33,919	92,506	37,087	101,146	96,57	3,54	84,70	33,07	85,96	38,591
165	35,966	98,089	36,101	98,457	34,386	93,780	36,409	99,297	97,41	2,47	85,43	36,30	87,19	43,974
166	33,149	90,406	34,713	94,672	36,149	98,588	35,861	97,803	95,37	3,71	83,65	28,48	85,75	37,661

150-50 4h GC 220 plus Triticale SSF

Duration SSF	Glucose					Xyl/Gal/Man				Arabinose			
[h]	HPLC	corrected	HPLC	corrected	mean [g/l]	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected
16	23,460	44,181	24,426	46,000	45,090	4,389	2,821	4,141	2,661	2,604	7,812	2,579	7,737
16,5	24,707	46,529	21,953	41,343	43,936	4,098	2,634	3,612	2,321	2,246	6,738	2,2	6,6
37,5	8,124	15,299	2,321	4,371	9,835	3,175	2,040	2,284	1,468	2,116	6,348	2,104	6,312
40	4,927	9,279	21,847	41,143	25,211	3,023	1,943	3,811	2,449	2,103	6,309	2,394	7,182
44	1,544	2,908	1,547	2,913	2,911	2,297	1,476	2,079	1,336	2,156	6,468	2,155	6,465
46	3,005	5,659	3,373	6,352	6,006	2,559	1,645	2,183	1,403	2,179	6,537	2,125	6,375
62	1,753	3,301	1,724	3,247	3,274	2,339	1,503	2,018	1,297	2,216	6,648	2,169	6,507
65	2,468	4,648	2,262	4,260	4,454	2,512	1,614	2,058	1,323	2,205	6,615	2,114	6,342
67	1,592	2,998	1,538	2,896	2,947	2,252	1,447	1,933	1,242	2,165	6,495	2,121	6,363
68	2,161	4,070	2,175	4,096	4,083	2,384	1,532	2,097	1,348	2,174	6,522	2,165	6,495
86	1,879	3,539	1,771	3,335	3,437	2,377	1,528	2,040	1,311	2,148	6,444	2,103	6,309
136	1,595	3,004	1,348	2,539	2,771	2,394	1,539	2,053	1,319	2,247	6,741	2,180	6,540
158	1,310	2,467	1,463	2,755	2,611	2,403	1,544	2,052	1,319	2,175	6,525	2,167	6,501

Duration SSF	Ethanol								total hydrolysed Glucan [%]	hydrolysed Cellulose [%]
[h]	HPLC	corrected	HPLC	corrected	mean [g/l]	Standard deviation	Yield [%]	Ethanol yield from Cellulose [%]		
16	2,566	6,415	2,131	5,328	5,871	0,769	4,11	5,20	-	-
16,5	2,068	5,170	2,571	6,428	5,799	0,889	4,06	5,14	-	-
37,5	12,278	30,695	14,754	36,885	33,790	4,377	23,65	29,95	-	-
40	14,560	36,400	3,264	8,160	22,280	19,969	15,60	19,75	-	-
44	17,677	44,193	17,429	43,573	43,883	0,438	30,72	38,90	-	-
46	17,270	43,175	16,235	40,588	41,881	1,830	29,32	37,12	-	-
62	21,702	54,255	21,23	53,075	53,665	0,834	37,57	47,57	-	-
65	21,106	52,765	22,199	55,498	54,131	1,932	37,89	47,98	-	-
67	22,657	56,643	21,929	54,823	55,733	1,287	39,01	49,40	-	-
68	22,386	55,965	22,880	57,200	56,583	0,873	39,61	50,15	-	-
86	25,98	64,950	25,287	63,218	64,084	1,225	44,86	56,80	-	-
136	32,925	82,313	34,509	86,273	84,293	2,800	59,00	74,72	-	-
158	35,772	89,430	35,761	89,403	89,416	0,019	62,59	79,26	4,65	10,26

150-50 23h GC 220 plus Triticale SSF

Duration SSF	Glucose					Xyl/Gal/Man				Arabinose			
[h]	HPLC	corrected	HPLC	corrected	mean [g/l]	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected
5	28,551	55,619	25,992	50,634	53,126	4,923	9,528	4,074	7,885	2,579	7,737	2,520	7,560
18,5	25,624	49,917	33,042	64,368	57,142	3,918	7,583	4,337	8,394	2,495	7,485	2,472	7,416
21	38,307	74,624	33,360	64,987	69,806	5,052	9,778	4,205	8,139	2,416	7,248	2,352	7,056
25	24,544	47,813	33,423	65,110	56,461	3,897	7,543	4,451	8,615	2,526	7,578	2,591	7,773
27	38,605	75,205	35,007	68,195	71,700	5,200	10,065	4,484	8,679	2,464	7,392	2,524	7,572
43	1,826	3,557	2,127	4,144	3,850	1,878	3,635	2,170	4,200	2,206	6,618	2,219	6,657
46	1,427	2,780	1,675	3,263	3,021	1,773	3,432	2,043	3,954	2,130	6,390	2,121	6,363
46	3,050	5,942	2,608	5,081	5,511	2,951	5,712	2,196	4,250	2,235	6,705	2,149	6,447
49	2,707	5,273	2,368	4,613	4,943	2,797	5,414	2,160	4,181	2,188	6,564	2,185	6,555
67	1,580	3,078	1,819	3,544	3,311	1,904	3,685	2,189	4,237	2,122	6,366	2,125	6,375
117	1,364	2,657	1,202	2,342	2,499	2,678	8,034	2,095	4,055	2,239	6,717	2,143	6,429
139	2,290	4,461	0,920	1,792	3,127	1,915	3,706	2,135	4,132	2,103	6,309	2,064	6,192

Duration SSF	Ethanol								total hydrolysed Glucan [%]	hydrolysed Cellulose [%]
[h]	HPLC	corrected	HPLC	corrected	mean [g/l]	Standard deviation	Yield [%]	Ethanol yield from Cellulose [%]		
5	0,393	0,983	0,436	1,090	1,036	0,076	0,92	-	24,93	-
18,5	2,137	5,343	1,676	4,190	4,766	0,815	4,22	-	30,06	-
21	1,612	4,030	2,020	5,050	4,540	0,721	4,02	-	35,58	-
25	4,164	10,410	3,097	7,743	9,076	1,886	8,05	-	33,57	-
27	2,169	5,423	2,954	7,385	6,404	1,388	5,68	-	38,09	-
43	17,764	44,410	19,503	48,758	46,584	3,074	41,29	-	43,03	-
46	18,796	46,990	20,645	51,613	49,301	3,269	43,70	-	45,07	-
46	18,706	46,765	18,216	45,540	46,153	0,866	40,91	-	43,40	-
49	19,847	49,618	19,002	47,505	48,561	1,494	43,04	-	45,28	-
67	23,638	59,095	25,771	64,428	61,761	3,771	54,74	-	56,24	-
117	35,765	89,413	34,597	86,493	87,953	2,065	77,96	-	79,09	3,88
139	35,956	89,890	37,352	93,380	91,635	2,468	81,22	13,69	82,64	20,19

150-50 24h GC 220 plus Triticale SSF

Duration SSF [h]	Glucose					Xyl/Gal/Man				Arabinose			
	HPLC	corrected	HPLC	corrected	mean [g/l]	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected
2	27,607	53,780	26,311	51,255	52,518	4,285	8,294	4,061	7,860	2,599	7,797	2,530	7,590
17,5	21,899	42,660	23,182	45,160	43,91	3,813	7,380	3,852	7,455	2,405	7,215	2,466	7,398
18	34,023	66,279	33,839	65,920	66,099	4,189	8,108	4,388	8,493	2,326	6,978	2,358	7,074
24	16,081	31,327	17,041	33,197	32,26	3,491	6,757	3,592	6,952	2,188	6,564	2,283	6,849
24	36,066	70,258	34,189	66,602	68,430	4,429	8,572	4,431	8,576	2,466	7,398	2,417	7,251
42	2,131	4,151	1,844	3,592	3,87	2,056	3,979	2,082	4,030	2,140	6,420	2,169	6,507
43	2,664	5,190	2,769	5,394	5,292	2,078	4,022	2,279	4,411	2,099	6,297	2,138	6,414
45	1,619	3,154	1,564	3,047	3,10	1,854	3,588	1,990	3,852	2,085	6,255	2,135	6,405
46	2,367	4,611	2,401	4,677	4,644	2,057	3,981	2,250	4,355	2,116	6,348	2,130	6,390
66	1,198	2,334	1,607	3,131	2,73	1,909	3,695	2,121	4,105	2,112	6,336	2,123	6,369
114	1,308	2,548	1,188	2,314	2,431	2,000	3,871	2,179	4,217	2,174	6,522	2,146	6,438
138	1,438	2,801	1,040	2,026	2,41	1,850	3,581	2,019	3,908	2,169	6,507	2,100	6,300

Duration SSF [h]	Ethanol								total hydrolysed Glucan [%]	hydrolysed Cellulose [%]
	HPLC	corrected	HPLC	corrected	mean [g/l]	Standard deviation	Yield [%]	Ethanol yield from Cellulose [%]		
2	0,242	0,605	0,187	0,468	0,536	0,097	0,48	-	24,22	-
17,5	4,784	11,960	4,684	11,710	11,835	0,177	10,49	-	30,34	-
18	1,354	3,385	1,557	3,893	3,639	0,359	3,23	-	33,11	-
24	8,481	21,203	8,602	21,505	21,354	0,214	18,93	-	33,51	-
24	2,005	5,013	1,981	4,953	4,983	0,042	4,42	-	35,35	-
42	17,611	44,028	18,613	46,533	45,280	1,771	40,14	-	41,89	-
43	16,502	41,255	16,797	41,993	41,624	0,521	36,89	-	39,29	-
45	19,045	47,613	19,710	49,275	48,444	1,176	42,94	-	44,34	-
46	20,786	51,965	18,284	45,710	48,838	4,423	43,29	-	45,39	-
66	23,267	58,168	23,805	59,513	58,840	0,951	52,16	-	53,39	-
114	34,436	86,090	33,705	84,263	85,176	1,292	75,50	-	76,60	-
138	35,524	88,810	34,351	85,878	87,344	2,074	77,42	-	78,51	-

6. The influence of the DS on the SSF process of corn silage and triticale as raw materials

160-30 21h GC 220 plus Triticale SSF 21,7%DS

Duration SSF [h]	Glucose										Xyl/Gal/Man								Arabinose							
	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected	mean [g/l]	Standard deviation	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected
3	29,474	58,948	28,015	56,03	27,78	55,56	29,015	58,03	57,14	1,61	5,448	10,896	5,117	10,234	5,526	11,052	5,304	10,608	3,098	9,294	3,062	9,186	2,96	8,88	3,025	9,075
4	32,047	64,094	30,142	60,284	30,174	60,348	32,473	64,946	62,42	2,45	6,004	12,008	5,938	11,876	5,949	11,898	5,951	11,902	3,179	9,537	3,219	9,657	3,244	9,732	3,089	9,267
21	33,797	67,594	35,543	71,086	36,767	73,534	37,713	75,426	71,91	3,38	6,372	12,744	6,213	12,426	6,438	12,876	6,638	13,276	3,056	9,168	3,146	9,438	3,146	9,438	3,198	9,594
22	35,6	71,2	33,912	67,824	33,048	66,096	32,588	65,176	67,57	2,65	6,068	12,136	5,583	11,166	6,077	12,154	5,73	11,46	3,182	9,546	3,112	9,336	2,987	8,961	3,004	9,012
45	33,549	67,098	35,828	71,656	35,586	71,172	38,485	76,97	71,72	4,05	6,517	13,034	6,498	12,996	6,38	12,76	6,817	13,634	2,948	8,844	3,109	9,327	2,983	8,949	3,077	9,231
51	32,02	64,04	35,338	70,676	32,26	64,52	35,702	71,404	67,66	3,92	6,175	12,35	6,436	12,872	6,019	12,038	6,401	12,802	3,057	9,171	3,039	9,117	2,956	8,868	3,074	9,222
70	2,159	4,318	9,248	18,496	2,391	4,782	2,652	5,304	8,23	6,86	3,357	6,714	4,548	9,096	3,324	6,648	3,482	6,964	2,712	8,136	3,067	9,201	2,72	8,16	2,779	8,337
73	1,593	3,186	1,522	3,044	1,793	3,586	1,682	3,364	3,30	0,23	2,834	5,668	2,597	5,194	3,11	6,22	2,796	5,592	2,659	7,977	2,56	7,68	2,65	7,95	2,605	7,815
94	4,668	9,336	2,01	4,02	2,322	4,644	2,064	4,128	5,53	2,55	3,319	6,638	2,709	5,418	3,238	6,476	2,857	5,714	2,611	7,833	2,582	7,746	2,725	8,175	2,67	8,01
118	1,273	2,546	1,226	2,452	1,593	3,186	1,438	2,876	2,77	0,33	2,831	5,662	2,542	5,084	3,024	6,048	2,826	5,652	2,604	7,812	2,5	7,5	2,687	8,061	2,61	7,83
121	1,316	2,632	1,461	2,922	1,704	3,408	1,987	3,974	3,23	0,59	3,31	6,62	3,174	6,348	3,187	6,374	3,398	6,796	2,782	8,346	2,735	8,205	2,746	8,238	2,915	8,745
141	1,209	2,418	1,169	2,338	1,613	3,226	1,542	3,084	2,77	0,45	2,871	5,742	2,636	5,272	3,104	6,208	2,715	5,43	2,678	8,034	2,541	7,623	2,719	8,157	2,563	7,689
142	1,429	2,858	1,603	3,206	1,743	3,486	1,888	3,776	3,33	0,39	3,323	6,646	3,302	6,604	3,187	6,374	3,277	6,554	2,807	8,421	2,789	8,367	2,729	8,187	2,846	8,538
150	1,036	2,072	1,143	2,286	1,612	3,224	1,849	3,698	2,82	0,77	3,181	6,362	2,938	5,876	3,509	7,018	3,152	6,304	2,387	7,161	2,35	7,05	2,493	7,479	2,47	7,41
165	1,206	2,412	1,319	2,638	2,349	4,698	2,975	5,95	3,92	1,70	3,174	6,348	2,906	5,812	3,406	6,812	3,051	6,102	2,39	7,17	2,317	6,951	2,469	7,407	2,436	7,308
166	1,374	2,748	1,912	3,824	2,668	5,336	3,436	6,872	4,70	1,80	3,139	6,278	3,152	6,304	3,195	6,39	3,387	6,774	2,66	7,98	2,787	8,361	2,761	8,283	2,831	8,493

Duration SSF [h]	Ethanol													
	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected	mean [g/l]	Standard deviation	Yield [%]	Ethanol yield from Cellulose [%]	total hydrolysed Glucan [%]	hydrolysed Cellulose [%]
3	0,327	0,892	0,264	0,720	0,293	0,799	0,304	0,829	0,81	0,07	0,71	-	26,27	-
4	0,489	1,334	0,227	0,619	0,166	0,453	0,238	0,649	0,76	0,39	0,67	-	28,59	-
21	1,514	4,129	0,764	2,084	0,846	2,307	0,759	2,070	2,65	0,99	2,32	-	34,49	-
22	1,186	3,235	1,255	3,423	1,333	3,635	1,462	3,987	3,57	0,32	3,13	-	33,36	-
45	3,329	9,079	2,839	7,743	3,150	8,591	3,084	8,411	8,46	0,55	7,42	-	39,50	-
51	4,236	11,553	3,173	8,654	4,381	11,948	4,007	10,928	10,77	1,47	9,45	-	39,71	-
70	20,097	54,810	16,071	43,830	21,418	58,413	21,922	59,787	54,21	7,23	47,55	-	51,23	-
73	26,232	71,542	26,258	71,613	25,721	70,148	27,298	74,449	71,94	1,80	63,10	-	64,57	-
94	20,479	55,852	22,399	61,088	22,015	60,041	23,229	63,352	60,08	3,14	52,70	-	55,17	-
118	30,485	83,141	29,657	80,883	29,159	79,525	31,197	85,083	82,16	2,46	72,06	-	73,30	-
121	32,396	88,353	31,958	87,158	34,475	94,023	35,929	97,988	91,88	5,05	80,59	15,10	82,03	21,427
141	33,218	90,595	32,467	88,546	31,298	85,358	33,859	92,343	89,21	3,00	78,25	4,86	79,48	10,270
142	30,056	81,971	29,763	81,172	32,057	87,428	31,662	86,351	84,23	3,12	73,88	-	75,37	-
150	35,326	96,344	35,298	96,267	33,919	92,506	37,087	101,146	96,57	3,54	84,70	33,07	85,96	38,591
165	35,966	98,089	36,101	98,457	34,386	93,780	36,409	99,297	97,41	2,47	85,43	36,30	87,19	43,974
166	33,149	90,406	34,713	94,672	36,149	98,588	35,861	97,803	95,37	3,71	83,65	28,48	85,75	37,661

160-30 18h GC 220 plus Triticale SSF 14%DS

Duration SSF [h]	Glucose						Xyl/Gal/Man				Arabinose			
	HPLC	corrected	HPLC	corrected	mean [g/l]	Standard deviation	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected
4,0	20,855	41,710	21,838	43,676	42,69	1,39	4,460	8,920	4,607	9,214	2,207	6,621	2,241	6,723
23,0	19,036	38,072	23,340	46,680	42,38	6,09	4,294	8,588	4,611	9,222	2,188	6,564	2,233	6,699
28,5	16,056	32,112	25,227	50,454	41,28	12,97	4,184	8,368	5,157	10,314	2,428	7,284	2,543	7,629
45,5	2,317	4,634	16,264	32,528	18,58	19,72	2,533	5,066	4,607	9,214	2,092	6,276	2,509	7,527
52,5	1,945	3,890	6,474	12,948	8,42	6,40	2,396	4,792	3,177	6,354	2,084	6,252	2,246	6,738
70,0	1,608	3,216	1,705	3,410	3,31	0,14	2,399	4,798	2,381	4,762	2,157	6,471	2,128	6,384
72,0	1,661	3,322	1,803	3,606	3,46	0,20	2,446	4,892	2,440	4,880	2,132	6,396	2,143	6,429
142,5	1,661	3,322	1,120	2,240	2,78	0,77	2,470	4,940	2,426	4,852	2,114	6,342	2,166	6,498

Duration SSF [h]	Ethanol								
	HPLC	corrected	HPLC	corrected	mean [g/l]	Standard deviation	Yield [%]	Ethanol yield from Cellulose [%]	total hydrolysed Glucan [%]	hydrolysed Cellulose [%]
4,0	0,749	1,765	0,766	1,805	1,79	0,03	1,82	-464,78	23,60	-
23,0	4,031	9,500	2,691	6,342	7,92	2,23	8,07	-428,82	29,69	-
28,5	6,591	15,533	3,354	7,904	11,72	5,39	11,94	-406,56	33,01	-
45,5	16,846	39,700	7,669	18,073	28,89	15,29	29,43	-305,93	38,92	-
52,5	19,097	45,005	14,493	34,155	39,58	7,67	40,33	-243,25	44,63	-
70,0	24,308	57,285	22,345	52,659	54,97	3,27	56,01	-153,03	57,70	-
72,0	24,217	57,071	22,779	53,682	55,38	2,40	56,43	-150,66	58,19	-
142,5	33,553	79,072	33,391	78,690	78,88	0,27	80,38	-12,89	81,79	-

160-30 120h GC 220 plus Triticale SSF 14,5%DS

Duration SSF [h]	Glucose										Xyl/Gal/Man								Arabinose							
	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected	mean [g/l]	Standard deviation	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected
18,0	18,670	56,010	23,574	70,722	24,618	73,854	28,076	84,228	71,20	11,66	4,576	13,728	5,231	15,693	5,428	16,284	5,457	16,371	2,033	6,099	2,530	7,590	2,290	6,870	2,701	8,103
25,0	11,795	35,385	21,558	64,674	23,297	69,891	30,739	92,217	65,54	23,39	3,952	11,856	5,231	15,693	5,009	15,027	5,607	16,821	2,075	6,225	2,540	7,620	2,527	7,581	2,831	8,493
42,0	1,751	5,253	2,227	6,681	4,723	14,169	32,766	98,298	31,10	44,97	2,470	7,410	2,844	8,532	3,358	10,074	5,644	16,932	2,136	6,408	2,213	6,639	2,364	7,092	2,674	8,022
49,0	1,723	5,169	1,917	5,751	1,992	5,976	31,182	93,546	27,61	43,96	2,457	7,371	2,746	8,238	2,616	7,848	6,113	18,339	2,219	6,657	2,376	7,128	2,287	6,861	2,923	8,769
66,0	1,497	4,491	1,606	4,818	1,712	5,136	1,945	5,835	5,07	0,57	2,387	7,161	2,664	7,992	2,543	7,629	2,766	8,298	2,210	6,630	2,388	7,164	2,316	6,948	2,387	7,161
73,0	1,507	4,521	1,595	4,785	1,650	4,950	1,740	5,220	4,87	0,29	2,359	7,077	2,658	7,974	2,501	7,503	2,569	7,707	2,235	6,705	2,445	7,335	2,268	6,804	2,264	6,792
90,5	1,330	3,990	1,508	4,524	1,618	4,854	1,668	5,004	4,59	0,45	2,316	6,948	2,674	8,022	2,538	7,614	2,801	8,403	2,266	6,798	2,532	7,596	2,345	7,035	2,595	7,785
92,5	1,436	4,308	1,464	4,392	1,702	5,106	1,671	4,713	4,63	0,36	2,264	6,792	2,633	7,599	2,509	7,527	2,565	7,695	2,161	6,483	2,239	6,717	2,245	6,736	2,352	7,056
163,0	1,379	4,137	1,379	4,137	1,965	5,895	1,537	4,611	4,70	0,83	2,775	8,325	2,775	8,325	2,605	7,815	2,695	8,085	2,520	7,560	2,520	7,560	2,411	7,233	2,389	7,167

Duration SSF [h]	Ethanol												
	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected	mean [g/l]	Standard deviation	Yield [%]	Ethanol yield from Cellulose [%]	total hydrolysed Glucan [%]	hydrolysed Cellulose [%]
18,0	3,162	9,486	2,206	6,618	1,608	4,824	0,751	2,253	5,80	3,04	5,29	-	38,44	-
25,0	7,201	21,603	4,137	12,411	3,074	9,222	0,970	2,910	11,54	7,79	10,53	-	41,04	-
42,0	15,915	47,745	14,673	44,019	13,180	39,540	1,737	5,211	34,13	19,57	31,15	-	45,63	-
49,0	17,865	53,595	17,454	52,362	17,198	51,594	2,947	8,841	41,60	21,85	37,97	-	50,83	-
66,0	20,707	62,121	20,749	62,247	20,682	62,046	18,093	54,279	60,17	3,93	54,93	-	57,29	-
73,0	21,764	65,292	21,751	65,253	21,719	65,157	19,345	58,035	63,43	3,60	57,91	-	60,17	-
90,5	24,771	74,313	25,430	76,290	25,817	77,451	26,318	78,954	76,75	1,96	70,06	-	72,20	-
92,5	23,939	71,817	23,359	70,077	24,599	73,797	23,099	69,297	71,25	2,00	65,04	-	67,19	-
163,0	33,947	101,841	33,940	101,820	35,378	106,134	33,688	101,064	102,71	2,31	93,76	64,13	95,95	76,70

160-30 18h GC 220 plus Triticale SSF 9,3%DS

Duration SSF [h]	Glucose						Xyl/Gal/Man				Arabinose			
	HPLC	corrected	HPLC	corrected	mean [g/l]	Standard deviation	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected
4,0	15,077	48,353	15,648	50,185	49,269	1,29	2,772	8,912	2,903	9,333	1,819	4,975	1,764	4,824
19,0	14,560	46,695	15,393	49,367	48,031	1,89	2,580	8,294	2,651	8,523	1,489	4,072	2,114	5,782
28,0	9,817	31,257	10,741	34,199	32,728	2,08	1,320	4,175	2,107	6,665	1,475	3,936	1,770	4,723
44,0	1,520	4,840	1,604	5,107	4,973	0,19	1,390	4,397	1,499	4,741	1,236	3,298	1,208	3,224
50,0	1,433	4,563	1,465	4,665	4,614	0,07	1,357	4,292	1,388	4,390	1,326	3,538	1,295	3,456
67,0	1,062	3,381	1,028	3,273	3,327	0,08	1,197	3,786	1,287	4,071	1,316	3,512	1,256	3,352
120,0	0,722	2,299	1,878	5,980	4,139	2,60	1,113	3,521	1,338	4,232	1,305	3,482	1,287	3,434
163,0	0,382	1,216	1,446	4,604	2,910	2,40	1,140	3,606	1,367	4,324	1,292	3,448	1,299	3,466
168,0	0,444	1,414	1,212	3,859	2,636	1,73	1,186	3,751	1,311	4,147	1,388	3,704	1,254	3,346
187,0	0,447	1,423	0,554	1,764	1,594	0,24	1,176	3,720	1,325	4,191	1,302	3,474	1,261	3,365
211,0	1,742	5,547	1,752	5,578	5,562	0,02	1,206	3,815	1,197	3,786	0,837	2,234	0,628	1,676

Duration SSF [h]	Ethanol								
	HPLC	corrected	HPLC	corrected	mean [g/l]	Standard deviation	Yield [%]	Ethanol yield from Cellulose [%]	total hydrolysed Glucan [%]	hydrolysed Cellulose [%]
4,0	0,513	1,693	0,518	1,709	1,70	3,04	1,86	-	29,32	-
19,0	2,984	9,847	2,969	9,798	9,82	2,29	10,74	-	37,51	-
28,0	6,637	20,515	7,095	21,930	21,22	5,60	23,19	-	41,44	-
44,0	14,423	44,581	15,867	49,044	46,81	1,36	51,16	-	53,93	-
50,0	17,812	55,056	17,997	55,628	55,34	1,46	60,48	-	63,06	-
67,0	20,552	63,525	20,412	63,093	63,31	2,02	69,19	-	71,05	-
120,0	27,466	84,896	25,754	79,605	82,25	5,76	89,89	-	92,20	37,20
163,0	28,817	89,072	26,965	83,348	86,21	5,51	94,22	-	95,84	66,53
168,0	31,065	96,021	26,701	82,532	89,28	11,24	97,57	-	99,04	92,28
187,0	28,672	88,624	27,396	84,680	86,65	4,73	94,70	-	95,59	64,51
211,0	28,899	89,326	27,287	84,343	86,83	3,52	94,90	-	98,00	83,92

160-30 18h GC 220 plus Triticale SSF 15%DS

Duration SSF [h]	Glucose						Xyl/Gal/Man				Arabinose			
	HPLC	corrected	HPLC	corrected	mean [g/l]	Standard deviation	HPLC	corrected	HPLC	corrected	HPLC	corrected	HPLC	corrected
4,0	18,603	59,662	18,934	60,723	60,192	0,751	3,507	11,274	3,322	10,680	2,613	7,146	2,754	7,532
19,0	20,146	64,610	20,995	67,333	65,972	1,925	3,592	11,548	3,534	11,361	2,720	7,439	2,725	7,453
28,0	21,051	67,027	23,067	73,445	70,236	4,539	3,309	10,467	2,946	9,319	2,501	6,674	2,646	7,061
44,0	18,895	60,162	21,096	67,170	63,666	4,955	3,219	10,182	3,152	9,970	2,732	7,290	2,772	7,397
50,0	13,653	43,471	12,715	40,485	41,978	2,112	3,358	10,622	3,155	9,980	2,616	6,981	2,365	6,311
67,0	1,265	4,028	1,277	4,066	4,047	0,027	1,956	6,187	1,824	5,769	1,868	4,985	1,886	5,033
68,0	0,964	3,069			3,069		1,750	5,535			1,917	5,116		
76,5	1,665	5,301			5,301		1,754	5,548			2,056	5,487		
92,0	3,752	11,946			11,946		1,897	6,000			1,960	5,230		
98,0	4,741	15,095			15,095		1,908	6,035			1,934	5,161		
116,0	6,827	21,737			21,737		1,980	6,263			1,960	5,230		
120,0	0,870	2,770	5,063	16,121	9,445	9,440	1,828	5,782	1,985	6,279	1,884	5,028	1,840	4,910
163,0	0,685	2,181	10,691	34,040	18,111	22,528	1,833	5,798	1,098	3,473	1,909	5,094	2,001	5,340
165,0	12,007	38,230			38,230		2,272	7,187			1,991	5,313		
168,0	0,649	2,066	11,477	36,543	19,305	24,379	1,784	5,643	2,134	6,750	1,891	5,046	2,016	5,380
187,0	0,707	2,251	12,990	41,360	21,806	27,654	1,780	5,630	2,082	6,586	1,880	5,017	1,968	5,252
211,0	0,950	3,025	15,358	48,900	25,962	32,439	1,794	5,675	2,253	7,126	1,912	5,102	2,032	5,422

Duration SSF [h]	Ethanol								total hydrolysed Glucan [%]	hydrolysed Cellulose [%]
	HPLC	corrected	HPLC	corrected	mean [g/l]	Standard deviation	Yield [%]	Ethanol yield from Cellulose [%]		
4,0	0,354	1,168	0,280	0,924	1,05	0,17	1,04		31,53	-
19,0	1,268	4,184	1,214	4,006	4,10	0,13	4,07		37,48	-
28,0	2,415	7,465	2,133	6,593	7,03	0,62	6,98		42,55	-
44,0	4,787	14,796	4,405	13,616	14,21	0,83	14,11		46,35	-
50,0	8,168	25,247	9,044	27,955	26,60	1,91	26,42		47,68	-
67,0	17,210	53,195	17,919	55,387	54,29	1,55	53,92		55,97	-
68,0	17,066	52,750			52,75		52,39		53,94	-
76,5	20,218	62,493			62,49		62,06		64,75	-
92,0	20,552	63,525			63,53		63,09		69,14	-
98,0	20,510	63,396			63,40		62,96		70,61	-
116,0	20,931	64,697			64,70		64,25		75,26	-
120,0	25,799	79,744	21,930	67,785	73,76	8,46	73,26		78,04	-
163,0	29,721	91,866	21,202	65,535	78,70	18,62	78,16		87,33	17,37
165,0	20,682	63,927			63,93		63,49		82,85	-
168,0	29,385	90,828	21,656	66,938	78,88	16,89	78,34		88,12	20,58
187,0	30,995	95,804	20,929	64,691	80,25	22,00	79,70		90,74	31,26
211,0	30,781	95,143	20,995	64,895	80,02	21,39	79,47		92,62	38,92

7. The formation of HMF and Furfural during the pre-treatment of corn silage using direct heating

Test	Glucose		Xylose		Arabinose		Lactic acid		Acetic acid		Ethanol		HMF		Furfural	
	HPLC [g/l]	corrected	HPLC [g/l]	corrected	HPLC [g/l]	corrected	HPLC [g/l]	corrected	HPLC [g/l]	corrected	HPLC [g/l]	corrected	HPLC [g/l]	corrected	HPLC [g/l]	corrected
135-30	0,069	0,690	0,030	0,300	0,146	1,460	0,143	1,430	0,622	6,220	0,208	2,080	0,000	0,000	0,000	0,000
135-40	0,090	0,900	0,045	0,450	0,142	1,420	0,126	1,260	0,072	0,720	0,044	0,440	0,000	0,000	0,001	0,010
140-30	0,058	0,580	0,036	0,360	0,099	0,990	0,396	3,960	0,180	1,800	0,055	0,550	0,000	0,000	0,000	0,000
140-40	0,107	1,070	0,037	0,370	0,091	0,910	0,023	0,230	0,019	0,190	0,048	0,480	0,000	0,000	0,000	0,000
140-90	0,174	1,740	0,106	1,060	0,280	2,800	0,144	1,440	0,158	1,580	0,008	0,080	0,002	0,020	0,000	0,000
145-30	0,056	0,560	0,032	0,320	0,106	1,060	0,132	1,320	0,254	2,540	0,000	0,000	0,000	0,000	0,000	0,000
145-40	0,081	0,810	0,043	0,430	0,132	1,320	0,121	1,210	0,434	4,340	0,056	0,560	0,001	0,010	0,002	0,020
145-70	0,120	1,200	0,084	0,840	0,243	2,430	0,114	1,140	0,118	1,180	0,046	0,460	0,000	0,000	0,000	0,000
145-90	0,180	1,800	0,108	1,080	0,251	2,510	0,193	1,930	0,124	1,240	0,000	0,000	0,000	0,000	0,000	0,000
150-30	0,058	0,580	0,031	0,310	0,104	1,040	0,312	3,120	0,092	0,920	0,000	0,000	0,000	0,000	0,000	0,000
150-40	0,060	0,600	0,040	0,400	0,096	0,960	0,069	0,690	0,308	3,080	0,000	0,000	0,000	0,000	0,000	0,000
150-50	0,117	1,170	0,069	0,690	0,208	2,080	0,057	0,570	0,057	0,570	0,000	0,000	0,000	0,000	0,002	0,020
150-60	0,119	1,190	0,039	0,390	0,090	0,900	0,103	1,030	0,080	0,800	0,000	0,000	0,001	0,010	0,001	0,010
150-70	0,122	1,220	0,100	1,000	0,243	2,430	0,055	0,550	0,114	1,140	0,000	0,000	0,002	0,020	0,000	0,000
150-90	0,157	1,570	0,127	1,270	0,262	2,620	0,098	0,980	0,186	1,860	0,000	0,000	0,000	0,000	0,002	0,020
155-30 ~10%TS	1,589	1,589	2,149	2,149	1,652	1,652	2,654	2,654	0,902	0,902	0,000	0,000	0,002	0,002	0,000	0,000
155-30	0,064	0,640	0,056	0,560	0,146	1,460	0,432	4,320	0,142	1,420	0,000	0,000	0,000	0,000	0,000	0,000
155-40	0,128	1,280	0,080	0,800	0,245	2,450	0,047	0,470	0,082	0,820	0,000	0,000	0,000	0,000	0,000	0,000
155-50	0,155	1,550	0,118	1,180	0,278	2,780	0,135	1,350	0,118	1,180	0,000	0,000	0,001	0,010	0,000	0,000
155-70	0,206	2,060	0,186	1,860	0,320	3,200	0,145	1,450	0,149	1,490	0,000	0,000	0,000	0,000	0,000	0,000
155-90	0,392	3,920	0,154	1,540	0,282	2,820	0,127	1,270	0,145	1,450	0,000	0,000	0,001	0,010	0,000	0,000
160-30	1,190	1,190	1,300	1,300	2,119	2,119	2,227	2,227	1,245	1,245	0,005	0,005	0,003	0,003	0,001	0,001

8. The production of bio-methane from different substrates

HBT 2005	Methan content (% vol.)	Specific gas yield (Nm3/kg oDS)	Specific methane yield (Nm3 CH4/kg oDS)	STD (%)	DS (% FM)	oDS (% DS)	Specific methane yield (Nm3 CH4/kg DS)
Maize silage dispesed (18 %DS)	52	0,683	0,353	2,0	18,28	97,47	0,344
Maize silage dispersed + Triticale (11 % DS)	53	0,638	0,335	3,9	10,86	94,19	0,316
Stillage from fermented and distilled (Maize silage + Triticale mash) + Straw (25 % DS)	52	0,599	0,310	1,6	24,48	94,60	0,293
HBT 2006							
Maize silage Meiereihof 05	52	0,707	0,376		33,23	96,83	0,364
Maize silage Meiereihof 05 dispersed	51	0,660	0,340		49,50	98,02	0,333
Maize silage Maiereihof + Triticale mash fermented and distillated	59	0,583	0,351		12,88	94,29	0,331
Triticale straw (milled with 10 mm sieve)	53	0,549	0,294		84,58	93,71	0,276
Triticale straw dispersed	52	0,619	0,329		7,29	94,23	0,310

9. The influence of the addition of stillage and urea with or without the addition of GC 106 respectively on the fermentation of triticale

adjunct	sample	experiment	FAN average extinction	FAN [mg/l]	average FAN [mg/l]	duration of fermentation [h]		
without adjunct	85	4.1	0,5878	540,91	180,68	20,50	18,38	
	86	4.2	0,1007	62,99		20,50		
	87	4.3	0,1104	72,51		16,25		
	88	4.4	0,0837	46,31		16,25		
	89	4.1	0,093	55,44	75,43	25,00	22,88	
	90	4.2	0,1125	74,57		25,00		
	91	4.3	0,1267	88,50		20,75		
	92	4.4	0,1213	83,20		20,75		
	93	4.1	0,107	69,17	187,70	44,25	42,13	
	94	4.2	0,1807	141,48		44,25		
	95	4.3	0,4175	373,82		40,00		
	96	4.4	0,206	166,31		40,00		
	97	4.1	0,1921	152,67	183,67	46,50	44,38	
	98	4.2	0,2076	167,88		46,50		
	99	4.3	0,2345	194,27		42,25		
	100	4.4	0,2606	219,88		42,25		
	101	4.1	0,1689	129,91	377,45	69,00	66,88	
	102	4.2	0,4584	413,95		69,00		
	103	4.3	0,5276	481,85		64,75		
	104	4.4	0,5299	484,11		64,75		
	105	4.1	0,4929	447,80	481,95	71,17	69,04	
	106	4.2	0,5784	531,69		71,17		
	107	4.3	0,4881	443,09		66,92		
	108	4.4	0,5514	505,20		66,92		
	109	4.1	0,4946	449,47	484,79	72,00	71,63	Blank value 0,04
	110	4.2	0,5107	465,27		72,00		
	111	4.3	0,6404	592,52		71,25		Standard 1,02
	112	4.4	0,4767	431,91		71,25		

adjunct	sample	experiment	FAN average extinction	FAN [mg/l]	average FAN [mg/l]	duration of fermentation [h]		
stillage	1	1.1	0,4977	452,51	201,06	19,17	17,33	
	2	1.2	0,1602	121,37		19,17		
	3	1.3	0,1545	115,78		15,50		
	4	1.4	0,1533	114,60		15,50		
	5	1.1	0,1642	125,29	105,47	23,83	21,67	
	6	1.2	0,1625	123,63		23,83		
	7	1.3	0,1243	86,15		19,50		
	8	1.4	0,125	86,83		19,50		
	9	1.1	0,1316	93,31	146,12	44,17	42,33	
	10	1.2	0,1519	113,23		44,17		
	11	1.3	0,1735	134,42		40,50		
	12	1.4	0,2847	243,52		40,50		
	13	1.1	0,229	188,87	217,57	47,17	45,33	
	14	1.2	0,2522	211,64		47,17		
	15	1.3	0,2256	185,54		43,50		
	16	1.4	0,3262	284,24		43,50		
	17	1.1	0,274	233,03	270,33	68,66	66,83	
	18	1.2	0,3162	274,43		68,66		
	19	1.3	0,2823	241,17		65,00		
	20	1.4	0,3756	332,71		65,00		
	21	1.1	0,2979	256,48	251,28	70,17	68,33	
	22	1.2	0,2472	206,73		70,17		
	23	1.3	0,314	272,27		66,50		
	24	1.4	0,3113	269,62		66,50		
	25	1.1	0,3364	294,25	264,47	72,00	72,00	Blank value 0,0365
	26	1.2	0,2937	252,35		72,00		
	27	1.3	0,2974	255,99		72,00		Standard 1,0192
	28	1.4	0,2967	255,30		72,00		

adjunct	sample	experiment	FAN average extinction	FAN [mg/l]	average FAN [mg/l]	duration of fermentation [h]		
1000mg/L Urea + GC 106	113	5.1	0,48	433,67	178,45	20,50	18,38	
	114	5.2	0,15	110,68		20,50		
	115	5.3	0,13	91,25		16,25		
	116	5.4	0,12	78,20		16,25		
	117	5.1	0,14	100,47	127,33	25,00	22,88	
	118	5.2	0,17	127,45		25,00		
	119	5.3	0,19	152,47		20,75		
	120	5.4	0,17	128,92		20,75		
	121	5.1	0,16	118,72	265,09	44,25	42,13	
	122	5.2	0,27	230,28		44,25		
	123	5.3	0,42	377,16		40,00		
	124	5.4	0,38	334,18		40,00		
	125	5.1	0,47	421,51	505,10	46,50	44,38	
	126	5.2	0,56	511,38		46,50		
	127	5.3	0,59	546,41		42,25		
	128	5.4	0,59	541,11		42,25		
	129	5.1	0,60	548,27	644,13	69,00	66,88	
	130	5.2	0,65	598,21		69,00		
	131	5.3	0,74	686,81		64,75		
	132	5.4	0,79	743,23		64,75		
	133	5.1	0,82	764,32	771,63	71,17	69,04	
	134	5.2	0,75	696,04		71,17		
	135	5.3	0,85	794,84		66,92		
	136	5.4	0,88	831,34		66,92		
	137	5.1	0,72	667,97	776,44	72,00	71,63	Blank value 0,0365
	138	5.2	0,79	738,52		72,00		
	139	5.3	0,77	722,33		71,25		Standard 1,0192
	140	5.4	1,03	976,94		71,25		

adjunct	sample	experiment	FAN average extinction	FAN [mg/l]	average FAN [mg/l]	duration of fermentation [h]		
500mg/L Urea + GC 106	29	2.1	0,27	230,28	113,96	19,17	17,33	
	30	2.2	0,10	62,21		19,17		
	31	2.3	0,09	49,06		15,50		
	32	2.4	0,15	114,31		15,50		
	33	2.1	0,14	98,12	65,96	23,83	21,67	
	34	2.2	0,12	79,47		23,83		
	35	2.3	0,07	36,70		19,50		
	36	2.4	0,09	49,55		19,50		
	37	2.1	0,09	55,93	120,88	44,17	42,33	
	38	2.2	0,14	103,61		44,17		
	39	2.3	0,17	129,51		40,50		
	40	2.4	0,23	194,47		40,50		
	41	2.1	0,22	180,63	253,29	47,17	45,33	
	42	2.2	0,21	172,19		47,17		
	43	2.3	0,27	230,77		43,50		
	44	2.4	0,47	429,55		43,50		
	45	2.1	0,34	301,12	369,11	68,66	66,83	
	46	2.2	0,37	322,80		68,66		
	47	2.3	0,43	387,85		65,00		
	48	2.4	0,51	464,68		65,00		
	49	2.1	0,46	419,05	386,75	70,17	68,33	
	50	2.2	0,33	289,34		70,17		
	51	2.3	0,40	356,26		66,50		
	52	2.4	0,53	482,34		66,50		
	53	2.1	0,51	462,52	443,39	72,00	72,00	Blank value 0,0365
	54	2.2	0,42	375,39		72,00		
	55	2.3	0,47	426,90		72,00		Standard 1,0192
	56	2.4	0,56	508,73		72,00		

adjunct	sample	experiment	FAN average extinction	FAN [mg/l]	average FAN [mg/l]	duration of fermentation [h]		
1000mg/L Urea	141	6.1	0,86	807,40	273,28	20,50	18,38	
	142	6.2	0,15	107,83		20,50		
	143	6.3	0,14	99,39		16,25		
	144	6.4	0,12	78,49		16,25		
	145	6.1	0,13	89,48	164,74	25,00	22,88	
	146	6.2	0,18	142,17		25,00		
	147	6.3	0,33	291,99		20,75		
	148	6.4	0,17	135,30		20,75		
	149	6.1	0,16	125,10	345,27	44,25	42,13	
	150	6.2	0,44	394,23		44,25		
	151	6.3	0,48	438,48		40,00		
	152	6.4	0,47	423,27		40,00		
	153	6.1	0,39	349,78	470,44	46,50	44,38	
	154	6.2	0,61	562,30		46,50		
	155	6.3	0,50	451,53		42,25		
	156	6.4	0,56	518,15		42,25		
	157	6.1	0,46	414,25	623,18	69,00	66,88	
	158	6.2	0,75	703,59		69,00		
	159	6.3	0,78	724,88		64,75		
	160	6.4	0,70	650,02		64,75		
	161	6.1	0,74	687,99	812,94	71,17	69,04	
	162	6.2	0,91	856,75		71,17		
	163	6.3	0,96	910,32		66,92		
	164	6.4	0,85	796,70		66,92		
	165	6.1	0,80	747,55	821,55	72,00	71,63	Blank value 0,0365
	166	6.2	0,87	822,41		72,00		
	167	6.3	0,99	931,22		71,25		Standard 1,0192
	168	6.4	0,84	785,03		71,25		

adjunct	sample	experiment	FAN average extinction	FAN [mg/l]	average FAN [mg/l]	duration of fermentation [h]		
500mg/L Urea	57	3.1	0,54	492,84	192,16	19,17	17,33	
	58	3.2	0,13	88,80		19,17		
	59	3.3	0,13	87,81		15,50		
	60	3.4	0,14	99,20		15,50		
	61	3.1	0,14	100,27	87,32	23,83	21,67	
	62	3.2	0,10	63,09		23,83		
	63	3.3	0,16	117,64		19,50		
	64	3.4	0,11	68,29		19,50		
	65	3.1	0,13	87,81	94,71	44,17	42,33	
	66	3.2	0,10	60,64		44,17		
	67	3.3	0,19	150,71		40,50		
	68	3.4	0,12	79,67		40,50		
	69	3.1	0,17	130,89	168,61	47,17	45,33	
	70	3.2	0,22	184,07		47,17		
	71	3.3	0,23	187,60		43,50		
	72	3.4	0,21	171,90		43,50		
	73	3.1	0,29	248,23	306,05	68,66	66,83	
	74	3.2	0,33	284,24		68,66		
	75	3.3	0,38	332,52		65,00		
	76	3.4	0,40	359,20		65,00		
	77	3.1	0,52	471,35	366,49	70,17	68,33	
	78	3.2	0,38	333,30		70,17		
	79	3.3	0,38	341,35		66,50		
	80	3.4	0,36	319,96		66,50		
	81	3.1	0,57	519,62	410,27	72,00	72,00	Blank value 0,0365
	82	3.2	0,32	278,85		72,00		
	83	3.3	0,42	378,24		72,00		Standard 1,0192
	84	3.4	0,51	464,38		72,00		

10. The fermentation of wheat using different temperatures and the dry substances

1,0g Thermosacc at 40°C (Water bath)

DS [%]	weighted wheat sample [g]	weight flask full [g]	Ethanol content [%vol.]	Ethanol yield [kg]/100kg FS	Ethanol yield [l]/100kg FS	Ethanol yield [l/100kg wheat]	Ethanol yield [%]	average	standard deviation	theoretical ethanol [%vol]	Density
20	53,71	250,10	8,73	69,09	70,05	41,21	97,6	98,45	1,25	8,95	986,3
	53,78	250,55	8,88	70,33	71,32	41,96	99,3			8,94	986,1
25	67,11	250,66	10,82	68,68	69,82	41,07	97,2	96,45	1,12	11,12	983,8
	67,11	250,78	10,64	67,58	68,68	40,40	95,7			11,12	984,0
30	80,64	262,88	10,82	59,98	60,93	35,84	84,9	82,92	2,74	12,76	984,5
	80,65	250,61	10,83	57,20	58,14	34,20	81,0			13,37	983,8
35	94,07	264,36	10,62	50,74	51,53	30,32	71,8	73,85	2,93	14,80	984,7
	94,16	274,70	10,82	53,68	54,50	32,06	75,9			14,26	984,9

1,0g Thermosacc at 40°C (Water bath)

DS [%]	weighted wheat sample [g]	weight flask full [g]	Ethanol content [%vol.]	Ethanol yield [kg]/100kg FS	Ethanol yield [l]/100kg FS	Ethanol yield [l/100kg wheat]	Ethanol yield [%]	average	standard deviation	theoretical ethanol [%vol]	Density
20	64,5	300,04	8,90	70,37	71,37	41,98	99,4	98,61	1,12	8,95	986,1
	64,5	300,00	8,76	69,26	70,23	41,31	97,8			8,96	986,2
25	80,6	300,00	10,84	68,58	69,72	41,02	97,1	98,47	1,92	11,16	983,7
	80,6	299,98	11,14	70,48	71,67	42,16	99,8			11,16	983,4
30	96,7	311,34	11,14	60,97	62,00	36,47	86,4	84,17	3,08	12,90	983,4
	96,7	300,00	10,98	57,90	58,87	34,63	82,0			13,39	983,6
35	112,8	333,27	10,86	54,54	55,44	32,62	77,2	72,53	6,64	14,06	983,7
	112,8	306,46	10,38	47,94	48,70	28,65	67,8			15,30	984,3

1,0g Thermosacc at 40°C (Inforce shaker 80rpm)

DS [%]	weighted wheat sample [g]	weight flask full [g]	Ethanol content [%vol.]	Ethanol yield [kg]/100kg FS	Ethanol yield [l]/100kg FS	Ethanol yield [l/100kg wheat]	Ethanol yield [%]	average	standard deviation	theoretical ethanol [%vol]	Density
20	64,5	300,54	8,72	69,09	70,06	41,22	97,6	97,52	0,09	8,94	986,1
	64,5	300,13	8,72	69,00	69,97	41,16	97,5			8,95	986,2
25	80,6	300,19	10,84	68,66	69,80	41,06	97,2	96,50	1,03	11,15	983,7
	80,6	300,09	10,68	67,62	68,76	40,45	95,8			11,15	983,4
30	96,7	300,03	11,44	60,33	61,35	36,09	85,5	84,35	1,57	13,39	983,4
	96,7	300,06	11,14	58,78	59,76	35,15	83,2			13,38	983,6
35	112,8	300,27	11,14	50,42	51,26	30,15	71,4	76,45	7,15	15,60	983,7
	112,8	330,01	11,58	57,59	58,51	34,42	81,5			14,21	984,3

1,0g Thermosacc at 30°C (Water bath)

DS [%]	weighted wheat sample [g]	weight flask full [g]	Ethanol content [%vol.]	Ethanol yield [kg]/100kg FS	Ethanol yield [l]/100kg FS	Ethanol yield [l/100kg wheat]	Ethanol yield [%]	average	standard *deviation*	theoretical ethanol *[%vol]*	Density
20	53,556	250,07	9,12	72,39	73,40	43,18	102,2	101,77	0,66	8,92	986,3
	53,555	255,86	8,83	71,72	72,73	42,79	101,3			8,72	986,1
25	67,133	250,58	11,35	72,00	73,19	43,06	101,9	102,23	0,40	11,13	983,8
	67,134	250,91	11,40	72,42	73,59	43,30	102,5			11,12	984,0
30	80,559	267,80	12,66	71,53	72,65	42,74	101,2	101,02	0,25	12,51	984,5
	80,559	274,60	12,29	71,23	72,40	42,59	100,8			12,19	983,8
35	93,987	275,04	11,17	55,56	56,43	33,20	78,6	80,91	3,27	14,21	984,7
	93,987	284,65	11,43	58,84	59,75	35,15	83,2			13,74	984,9

FS [%] 58,83

11. The fermentation of maize using different temperatures and the dry substances

1g Thermosacc at 40°C (water bath)								
%DS	weigted maize sample [g]	weight flask full [g]	Ethanol content [%vol.]	Ethanol yoield [kg/100kg FS]	Ethanol yield [l/100kg FS]	Ethanol yield [%]	average [%]	standard deviation
20	55,50	250,31	8,11	56,27	57,16	78,28	78,49	0,297
	55,50	254,82	8,30	57,59	58,50	78,70		
25	69,40	250,30	8,56	47,52	48,31	66,11	66,19	0,108
	69,39	255,22	8,75	48,57	49,37	66,27		
30	83,30	267,83	8,69	40,18	40,82	52,24	54,75	3,552
	83,25	257,70	9,16	42,38	43,10	57,27		
35	97,12	266,83	8,48	33,62	34,14	43,87	43,15	1,026
	97,13	279,90	8,60	34,10	34,62	42,42		

1g Thermosacc at 30°C (water bath)								
%DS	weigted maize sample [g]	weight flask full [g]	Ethanol content [%vol.]	Ethanol yoield [kg/100kg FS]	Ethanol yield [l/100kg FS]	Ethanol yield [%]	average [%]	standard deviation
20	55,50	255,58	9,34	66,24	67,41	95,17	95,30	0,18
	55,50	250,28	9,34	64,89	66,04	95,43		
25	69,40	260,88	10,84	62,76	63,80	91,07	93,73	3,76
	69,40	250,19	11,44	63,50	64,60	96,39		
30	83,30	286,44	11,73	62,15	63,25	72,11	77,73	7,96
	83,30	266,70	12,47	61,50	62,64	83,36		
35	97,10	287,48	11,96	54,55	55,52	65,48	65,24	0,34
	97,10	287,04	11,99	54,61	55,59	65,00		

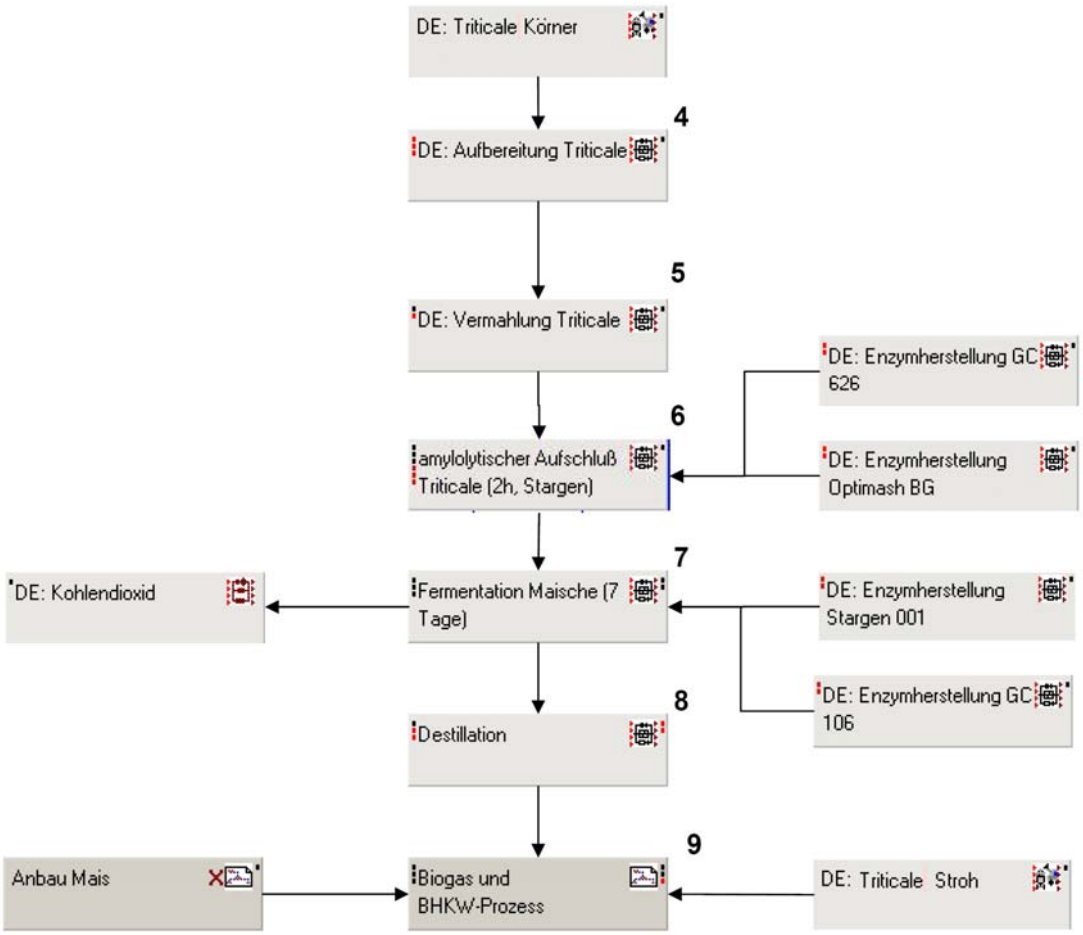
12. The influence of the DS on the ethanol yield during an SSF process using the stargen™ enzymes

Dry Substance [%]	Fermentation time [h]	Ethanol content [g/l]	Yield from theory [%]	Yield from 100kg raw material [%]	Hydrolysed glucane [%]	resulting glucane [%]
25	18,3	36,49	36,27	16,80	37,61	62,39
	26,0	47,63	47,35	21,93	48,14	51,86
	42,5	65,62	65,23	30,21	65,41	34,59
	49,0	72,59	72,16	33,42	72,17	27,83
	66,0	86,16	85,64	39,67	85,34	14,66
	72,5	86,77	86,26	39,95	85,49	14,51
	89,0	94,54	93,98	43,53	93,17	6,83
	93,0	99,72	99,12	45,91	100,15	-0,15
	2,0	2,17	2,16	1,00	16,08	83,92
	17,0	27,50	27,34	12,66	28,64	71,36
	25,0	38,64	38,41	17,79	39,10	60,90
	41,0	55,31	54,98	25,46	55,16	44,84
	48,0	63,57	63,19	29,27	63,15	36,85
	65,0	76,31	75,86	35,13	75,50	24,50
	73,0	80,40	79,92	37,02	79,33	20,67
	89,0	92,40	91,85	42,54	91,10	8,90
	93,0	93,91	93,35	43,24	92,54	7,46
31	18,3	42,24	33,79	15,65	35,17	64,83
	26,0	55,25	44,20	20,47	45,09	54,91
	42,5	78,06	62,44	28,92	62,60	37,40
	49,0	87,14	69,71	32,29	69,63	30,37
	66,0	101,43	81,14	37,58	81,28	18,72
	72,0	102,57	82,06	38,00	82,51	17,49
	89,0	109,62	87,69	40,61	89,07	10,93
	93,0	101,51	81,20	37,61	81,38	18,62
	2,0	2,53	2,02	0,94	16,43	83,57
	17,0	33,30	26,64	12,34	27,70	72,30
	25,0	46,68	37,34	17,30	37,75	62,25
	41,0	65,72	52,58	24,35	52,51	47,49
	48,0	76,79	61,43	28,45	61,18	38,82
	65,0	90,68	72,54	33,60	72,08	27,92
	73,0	97,06	77,64	35,96	76,66	23,34
	89,0	106,25	85,00	39,37	85,03	14,97
	93,0	112,06	89,65	41,52	89,69	10,31
37	18,3	37,21	29,68	13,75	30,39	69,61
	26,0	46,33	38,67	17,91	38,88	61,12
	42,5	61,40	55,21	25,57	54,95	45,05
	49,0	65,68	60,35	27,95	60,63	39,37
	66,0	73,38	69,01	31,96	70,15	29,85
	72,0	72,57	67,31	31,17	69,09	30,91
	89,0	73,48	71,10	32,93	73,98	26,02
	93,0	75,17	71,06	32,91	74,08	25,92
	2,0	2,38	1,63	0,76	16,05	83,95
	17,0	29,40	20,14	9,33	21,31	78,69
	25,0	51,27	35,11	16,26	35,58	64,42
	41,0	72,66	49,77	23,05	49,83	50,17
	48,0	83,37	57,10	26,45	57,36	42,64
	65,0	96,90	66,37	30,74	68,34	31,66
	73,0	99,36	68,05	31,52	71,56	28,44
	89,0	109,64	75,10	34,78	80,61	19,39
	93,0	109,43	74,95	34,71	80,94	19,06

Dry Substance [%]	Yield after 93 h fermentation [%]	Average yield [%]	Standard deviation
25,17	99,12	96,24	4,08
	93,35		
31,47	81,20	85,43	5,97
	89,65		
36,86	71,06	73,01	2,75
	74,95		

13. Ecobalance

Distillery run with cereals to produce ethanol



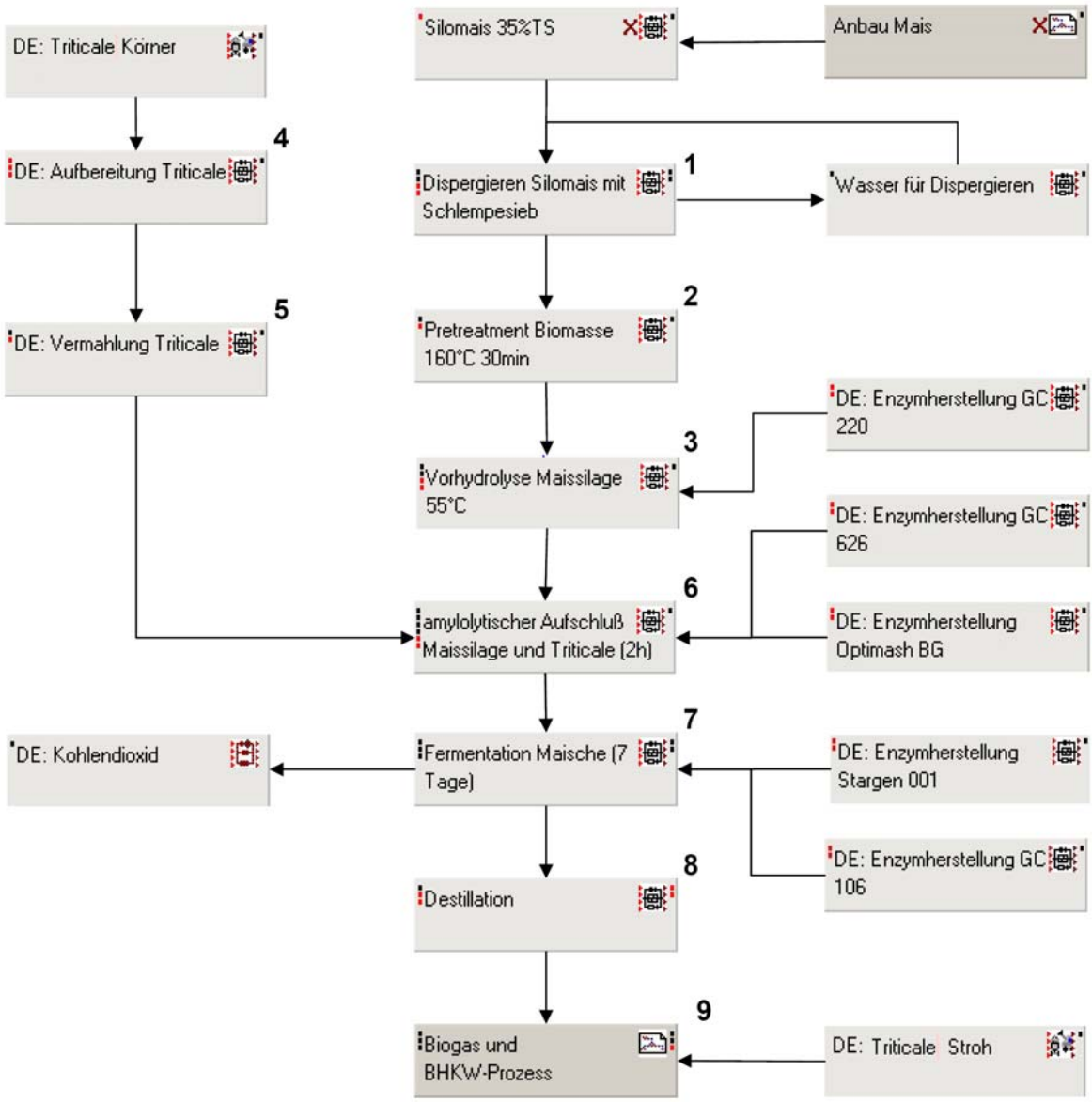
Distillery feed with autarcic energy supply

Nummer im Flowchart/Number in flow chart				
4 Aufbereitung Triticale (conditioning of triticale)	**Inputs**	**Inputs**		
	Electricity	Strom	52,611 MJ	entnommen aus Studie Senn
	Thermal energy	thermische Energie	136,28 MJ	
	Triticale	Triticale	1023,8 kg	
	Outputs	**Outputs**		
	Waste heat	Abwärme	242,77 MJ	
	Water vapour	Wasserdampf	23,807 kg	
	Triticale	Triticale	1000 kg	
5 Vermahlung Triticale (milling of triticale)	**Inputs**	**Inputs**		
	Triticale	Triticale	1000 kg	
	Electricity	Strom	22 MJ	22MJ/t entnommen aus Studie Senn
	Outputs	**Outputs**		
	Milled Triticale	Triticale vermahlen	1000 kg	
6 Amylolytischer Aufschluß Triticale (2h) (amylolytic hydrolysis of triticale)	**Inputs**	**Inputs**		
	GC 626	GC 626	$160{,}7 * 10^{-6}$ kg	Angabe Enzymhersteller
	Opimasch BG	Opimasch BG	$32{,}14 * 10^{-6}$ kg	Angabe Enzymhersteller
	Electricity	Strom	164,79 MJ	Hochrechung aus Maischversuchen
	Thermal energy	thermische Energie	130,63 MJ	
	Milled Triticale	Triticale vermahlen	1000 kg	
	Water	Wasser	2571,4 kg	
	Outputs	**Outputs**		
	Saccharified mash	verzuckerte Maische	3571,4 kg	
7 Fermentation (fermentation)	**Inputs**	**Inputs**		
	Saccharified mash	verzuckerte Maische	3571,4 kg	
	Yeast	Hefe	19,592 kg	
	GC 106	GC 106	$26{,}36 * 10^{-6}$ kg	Angabe Enzymhersteller
	Stargen 001	Stargen 001	0,3909 kg	Angabe Enzymhersteller
	Outputs	**Outputs**		
	Carbon dioxide	Kohlendioxid	336,04 kg	stöchiometrische Berechnung
	Fermented mash	vergorene Maische	3571,4 kg	Berechnung
8 Destillation (distillation)	**Inputs**	**Inputs**		
	Fermented mash	vergorene Maische	3571,4 kg	
	Electricity	Strom	98,89 MJ	Studie Senn
	Thermal energy	thermische Energie	1943,7 MJ	Studie Senn
	Outputs	**Outputs**		
	Ethanol	Ethanol	351,78 kg	Hochrechnung aus Maischversuchen
	Stillage	Schlempe	3219,7 kg	Berechnung
9 Biogasprozess (biogas process)	**Inputs**	**Inputs**		
	Stillage	Schlempe	3219,7 kg	Berechnung
	Maize sillage 35%DS	Silomais 35%TS	3475 kg	Berechnung
	Triticale straw 90%DS	Triticalestroh 90%TS	1023,8 kg	Berechnung
	Outputs	**Outputs**		
	Biogas	Biogas	930,74 kg	Berechnung aus Biogasversuchen Schlempe 10,8% TS [0,29598 m3 CH4/kg TS] Silomais [0,344m3 CH4/kg TS] Triticalestroh [0,276 m3 CH4/kg TS]
	Electricity	Strom	1657,9 MJ	30% Wirkungsgrad Quelle: Handreichung Biogas Model 1 Seite 207
	Thermal energy	thermische Energie	2210,5 MJ	40% Wirkungsgrad Quelle: Handreichung Biogas Model 1 Seite 207

Distillery with fossil energy supply

Nummer im Flowchart/Number in flow chart				
4 Aufbereitung Triticale (conditioning of triticale)	**Inputs**	**Inputs**		
	Electricity	Strom	52,611 MJ	entnommen aus Studie Senn
	Thermal energy	thermische Energie	136,28 MJ	
	Triticale	Triticale	1023,8 kg	
	Outputs	**Outputs**		
	Waste heat	Abwärme	242,77 MJ	
	Water vapour	Wasserdampf	23,807 kg	
	Triticale	Triticale	1000 kg	
5 Vermahlung Triticale (milling of triticale)	**Inputs**	**Inputs**		
	Triticale	Triticale	1000 kg	
	Electricity	Strom	22 MJ	22MJ/t entnommen aus Studie Senn
	Outputs	**Outputs**		
	Milled Triticale	Triticale vermahlen	1000 kg	
6 Amylolytischer Aufschluß Triticale (2h) (amylolytic hydrolysis of triticale)	**Inputs**	**Inputs**		
	GC 626	GC 626	$160,7 * 10^{-6}$ kg	Angabe Enzymhersteller
	Opimasch BG	Opimasch BG	$32,14 * 10^{-6}$ kg	Angabe Enzymhersteller
	Electricity	Strom	164,79 MJ	Hochrechung aus Maischversuchen
	Thermal energy	thermische Energie	130,63 MJ	
	Milled Triticale	Triticale vermahlen	1000 kg	
	Water	Wasser	2571,4 kg	
	Outputs	**Outputs**		
	Saccharified mash	verzuckerte Maische	3571,4 kg	
7 Fermentation (fermentation)	**Inputs**	**Inputs**		
	Saccharified mash	verzuckerte Maische	3571,4 kg	
	Yeast	Hefe	19,592 kg	
	GC 106	GC 106	$26,36 * 10^{-6}$ kg	Angabe Enzymhersteller
	Stargen 001	Stargen 001	0,3909 kg	Angabe Enzymhersteller
	Outputs	**Outputs**		
	Carbon dioxide	Kohlendioxid	336,04 kg	stöchiometrische Berechnung
	Fermented mash	vergorene Maische	3571,4 kg	Berechnung
8 Destillation (distillation)	**Inputs**	**Inputs**		
	Fermented mash	vergorene Maische	3571,4 kg	
	Electricity	Strom	98,89 MJ	Studie Senn
	Thermal energy	thermische Energie	1943,7 MJ	Studie Senn
	Outputs	**Outputs**		
	Ethanol	Ethanol	351,78 kg	Hochrechnung aus Maischversuchen
	Stillage	Schlempe	3219,7 kg	Berechnung
9 Biogasprozess (biogas process)	**Inputs**	**Inputs**		
	Stillage	Schlempe	3219,7 kg	Berechnung
	Maize sillage 35%DS	Silomais 35%TS	3475 kg	Berechnung
	Triticale straw 90%DS	Triticalestroh 90%TS	1023,8 kg	Berechnung
	Outputs	**Outputs**		
	Biogas	Biogas	1167,9 kg	Berechnung aus Biogasversuchen Schlempe 10,8% TS [0,29598 m3 CH4/kg TS] Silomais [0,344m3 CH4/kg TS] Triticalestroh [0,276 m3 CH4/kg TS]

Combined distillery run with maize silage and triticale to produce ethanol



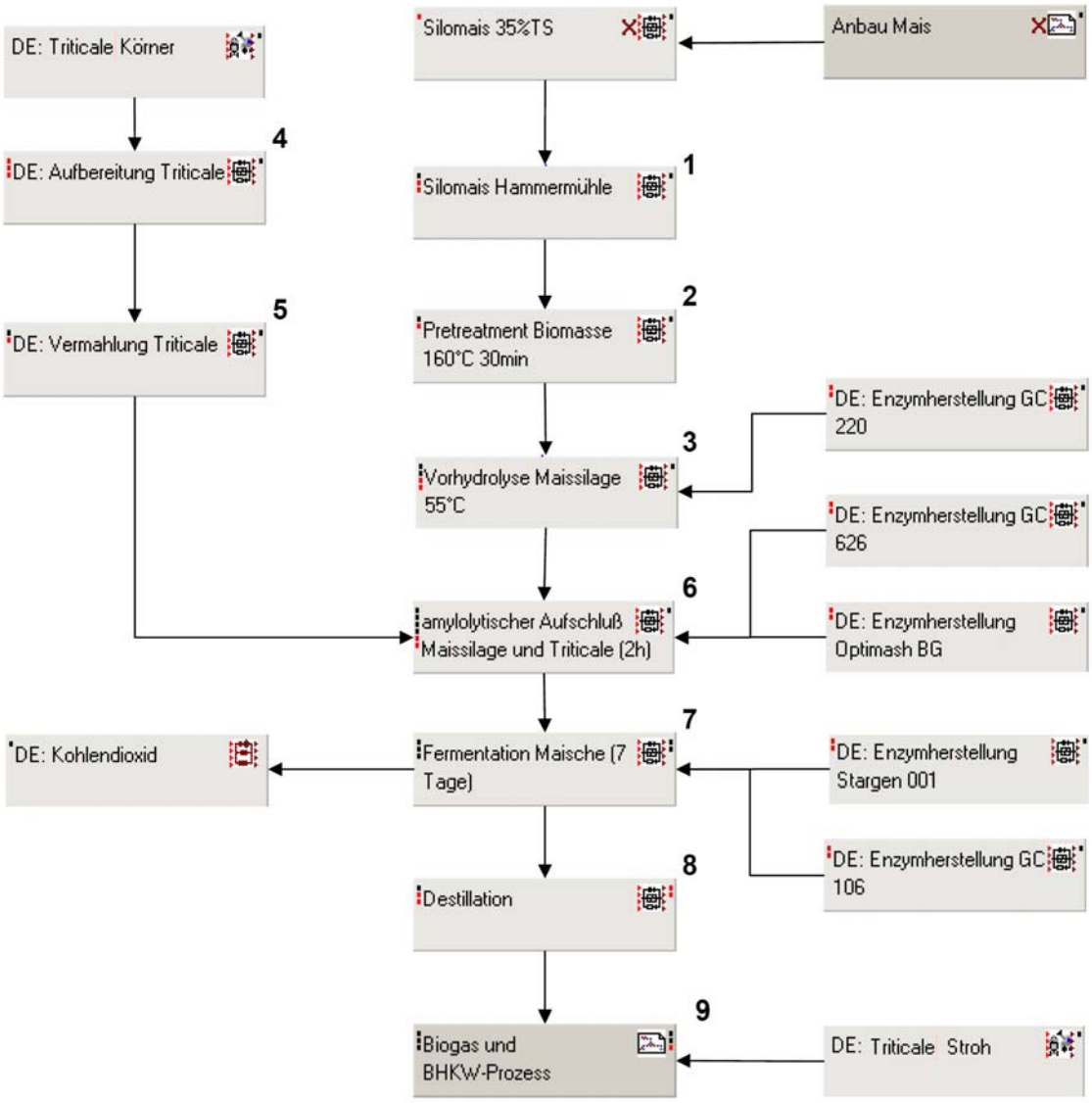
Distillery with autarcic energy supply

Nummer im Flowchart/Number in flow chart				
1 dispergieren Silomais mit Schlempesieb (dispersing of maize silage with stillage sieve)	**Inputs**	**Inputs**		
	Maize silage 35%DS	Silomais 35%TS	100 kg	
	Electricity	Strom	21,18 MJ	gemessen mit Becomix-Homogenisator
	Water for dispersing	Wasser für Dispergieren	70 kg	gewogen
	Water	Wasser	135 kg	gewogen
	Outputs	**Outputs**		
	Dispersed maize silage	Dispergierte Maissilage	170 kg	
	Water from dispersing	Wasser aus Dispergieren	135 kg	gewogen
2 Pretreatment Biomasse 160°C 30min (pretreatment of biomass 160°C 30min)	**Inputs**	**Inputs**		
	Dispersed maize silage	Dispergierte Maissilage	170 kg	
	Thermal energy	thermische Energie	79,61 MJ	Berechnung: Erhitzen von 144,5 kg Wasser (4,1855J/(g*k)) und 25,5kg Stärke (2,3J/(g*k)) um 120k
	Outputs	**Outputs**		
	Thermal pretreated maize silage	thermisch vorbehandelte Maissilage	145,69 kg	Hochrechnung aus Kleinversuchen
	Water vapour	Wasserdampf	24,31 kg	Berechnung uas Kleinversuchen
3 Vorhydrolyse Maissilage 55°C (prehydrolysis of maize silage 55°C)	**Inputs**	**Inputs**		
	Cellulase	Cellulase	5,2448 kg	Hochrechnung aus Kleinversuchen
	Electricity	Strom	14,394 MJ	6mal Rühren für 1h innerhalb 24h (Energieverbrauch für Rühren
	Thermal pretreated maize silage	thermisch vorbehandelte Maissilage	145,69 kg	
	Thermal energy	thermische Energie	6,0979 MJ	Nachheizen der Maische um 10°C innerhalb 24h
	Outputs	**Outputs**		
	Maize silage saccharified	Maissilagemaische verzuckert	145,69 kg	
4 Aufbereitung Triticale (conditioning of triticale)	**Inputs**	**Inputs**		
	Electricity	Strom	2,146 MJ	entnommen aus Studie Senn
	Thermal energy	thermische Energie	5,559 MJ	
	Triticale	Triticale	41,764 kg	
	Outputs	**Outputs**		
	Waste heat	Abwärme	9,903 MJ	
	Water vapour	Wasserdampf	0,97 kg	
	Triticale	Triticale	40,793 kg	
5 Vermahlung Triticale (milling of triticale)	**Inputs**	**Inputs**		
	Triticale	Triticale	40,7932 kg	
	Electricity	Strom	0,897 MJ	22MJ/t entnommen aus Studie Senn
	Outputs	**Outputs**		
	Milled Triticale	Triticale vermahlen	40,7932 kg	
6 Amylolytischer Aufschluß Triticale (2h) (amylolytic hydrolysis of triticale)	**Inputs**	**Inputs**		
	GC 626	GC 626	$6{,}556 * 10^{-5}$ kg	Angabe Enzymhersteller
	Opimasch BG	Opimasch BG	$1{,}311 * 10^{-5}$ kg	Angabe Enzymhersteller
	Electricity	Strom	6,7221 MJ	Hochrechung aus Maischversuchen
	Thermal energy	thermische Energie	7,7186 MJ	
	Milled Triticale	Triticale vermahlen	40,793 kg	
	Outputs	**Outputs**		
	Saccharified mash	verzuckerte Maische	186,48 kg	
7 Fermentation (fermentation)	**Inputs**	**Inputs**		
	Saccharified mash	verzuckerte Maische	186,48 kg	
	Yeast	Hefe	0,79921 kg	
	GC 106	GC 106	$1{,}075 * 10^{-5}$ kg	Angabe Enzymhersteller
	Stargen 001	Stargen 001	0,0159 kg	Angabe Enzymhersteller
	Outputs	**Outputs**		
	Carbon dioxide	Kohlendioxid	17,281 kg	stöchiometrische Berechnung
	Fermented mash	vergorene Maische	169,2 kg	Berechnung
8 Destillation (distillation)	**Inputs**	**Inputs**		
	Fermented mash	vergorene Maische	169,2 kg	
	Electricity	Strom	5,16 MJ	Studie Senn
	Thermal energy	thermische Energie	100,49 MJ	Studie Senn
	Outputs	**Outputs**		
	Ethanol	Ethanol	18,09 kg	Hochrechnung aus Maischversuchen
	Stillage	Schlempe	151,11 kg	Berechnung
9 Biogasprozess (biogas process)	**Inputs**	**Inputs**		
	Stillage	Schlempe	151,11 kg	Berechnung
	Maize silage 35%DS	Silomais 35%TS	41,758 kg	Berechnung
	Triticale straw 90%DS	Triticalestroh 90%TS	41,765 kg	Berechnung
	Outputs	**Outputs**		
	Biogas	Biogas	28,264 kg	Berechnung aus Biogasversuchen 14,615kg TS aus Schlempe [0,344m3 CH4/kg TS] und 34,674kg TS aus Triticalestroh [0,276 m3 CH4/kg TS]
	Electricity	Strom	149,67 MJ	30% Wirkungsgrad Quelle: Handreichung Biogas Model 1 Seite 207
	Thermal energy	thermische Energie	199,57 MJ	40% Wirkungsgrad Quelle: Handreichung Biogas Model 1 Seite 207

Distillery with fossil energy supply

Nummer im Flowchart/ number in flow chart				
1 dispergieren Silomais mit Schlempesieb (dispersing of maize silage with stillage sieve)	**Inputs**	**Inputs**		
	Maize silage 35%DS	Silomais 35%TS	100 kg	
	Electricity	Strom	21,18 MJ	gemessen mit Becomix-Homogenisator
	Water for dispersing	Wasser für Dispergieren	70 kg	gewogen
	Water	Wasser	135 kg	gewogen
	Outputs	**Outputs**		
	Dispersed maize silage	Dispergierte Maissilage	170 kg	
	Water from dispersing	Wasser aus Dispergieren	135 kg	gewogen
2 Pretreatment Biomasse 160°C 30min (pretreatment of biomass 160°C 30min)	**Inputs**	**Inputs**		
	Dispersed maize silage	Dispergierte Maissilage	170 kg	
	Thermal energy	thermische Energie	79,61 MJ	Berechnung: Erhitzen von 144,5 kg Wasser (4,1855J/(g*k)) und 25,5kg Stärke (2,3J/(g*k)) um 120k
	Outputs	**Outputs**		
	Thermal pretreated maize silage	thermisch vorbehandelte Maissilage	145,69 kg	Hochrechnung aus Kleinversuchen
	Water vapour	Wasserdampf	24,31 kg	Berechnung uas Kleinversuchen
3 Vorhydrolyse Maissilage 55°C (prehydrolysis of maize silage 55°C)	**Inputs**	**Inputs**		
	Cellulase	Cellulase	5,2448 kg	Hochrechnung aus Kleinversuchen
	Electricity	Strom	14,394 MJ	6mal Rühren für 1h innerhalb 24h (Energieverbrauch für Rühren bei Maischversuchen gemessen)
	Thermal pretreated maize silage	thermisch vorbehandelte Maissilage	145,69 kg	
	Thermal energy	thermische Energie	6,0979 MJ	Nachheizen der Maische um 10°C innerhalb 24h
	Outputs	**Outputs**		
	Maize silage saccharified	Maissilagemaische verzuckert	145,69 kg	
4 Aufbereitung Triticale (conditioning of triticale)	**Inputs**	**Inputs**		
	Electricity	Strom	2,146 MJ	entnommen aus Studie Senn
	Thermal energy	thermische Energie	5,559 MJ	
	Triticale	Triticale	41,764 kg	
	Outputs	**Outputs**		
	Waste heat	Abwärme	9,903 MJ	
	Water vapour	Wasserdampf	0,97 kg	
	Triticale	Triticale	40,793 kg	
5 Vermahlung Triticale (milling of triticale)	**Inputs**	**Inputs**		
	Triticale	Triticale	40,7932 kg	
	Electricity	Strom	0,897 MJ	22MJ/t entnommen aus Studie Senn
	Outputs	**Outputs**		
	Milled Triticale	Triticale vermahlen	40,7932 kg	
6 Amylolytischer Aufschluß Maissilage und Triticale (2h) (amylolytic hydrolysis of maize silage and triticale)	**Inputs**	**Inputs**		
	GC 626	GC 626	$6{,}556 * 10^{-6}$ kg	Angabe Enzymhersteller
	Opimasch BG	Opimasch BG	$1{,}311 * 10^{-5}$ kg	Angabe Enzymhersteller
	Electricity	Strom	6,7221 MJ	Hochrechung aus Maischversuchen
	Thermal energy	thermische Energie	7,7186 MJ	
	Milled Triticale	Triticale vermahlen	40,793 kg	
	Outputs	**Outputs**		
	Saccharified mash	verzuckerte Maische	186,48 kg	
7 Fermentation (fermentation)	**Inputs**	**Inputs**		
	Saccharified mash	verzuckerte Maische	186,48 kg	
	Yeast	Hefe	0,79921 kg	
	GC 106	GC 106	$1{,}075 * 10^{-6}$ kg	Angabe Enzymhersteller
	Stargen 001	Stargen 001	0,0159 kg	Angabe Enzymhersteller
	Outputs	**Outputs**		
	Carbon dioxide	Kohlendioxid	17,281 kg	stöchiometrische Berechnung
	fermented mash	vergorene Maische	169,2 kg	Berechnung
8 Destillation (distillation)	**Inputs**	**Inputs**		
	fermented mash	vergorene Maische	169,2 kg	
	Electricity	Strom	5,16 MJ	Studie Senn
	Thermal energy	thermische Energie	100,49 MJ	Studie Senn
	Outputs	**Outputs**		
	Ethanol	Ethanol	18,09 kg	Hochrechnung aus Maischversuchen
	Stillage	Schlempe	151,11 kg	Berechnung
9 Biogasprozess (biogas process)	**Inputs**	**Inputs**		
	Stillage	Schlempe	151,11 kg	Berechnung
	Maize silage 30%DS	Silomais 35%TS	41,758 kg	Berechnung
	Triticale straw 90%DS	Triticalestroh 90%TS	41,765 kg	Berechnung
	Outputs	**Outputs**		
	Biogas	Biogas	50,884 kg	Berechnung aus Biogasversuchen 14,615kg TS aus Schlempe [0,344m3 CH4/kg TS] und 34,674kg TS aus Triticalestroh [0,276 m3 CH4/kg TS]

Combined distillery run with hammermilled maize silage and triticale to produce ethanol



Distillery with autarcic energy supply

Nummer im Flowchart/Number in flow chart				
1 Silomais Hammermühle (maize silage hammermilled)	**Inputs**	**Inputs**		
	Maize silage 35%DS	Silomais 35%TS	100 kg	
	Water	Wasser	70 kg	gewogen
	Electricity	Strom	9 MJ	Leitfaden Bioenergie 25kWh/t
	Outputs	**Outputs**		
	Dispersed maize silage	Dispergierte Maissilage	170 kg	
2 Pretreatment Biomasse 160°C 30min (pretreatment of biomass 160°C 30min)	**Inputs**	**Inputs**		
	Dispersed maize silage	Dispergierte Maissilage	170 kg	
	Thermal energy	thermische Energie	79,61 MJ	Berechnung: Erhitzen von 144,5 kg Wasser (4,1855J/(g*k)) und 25,5kg Stärke (2,3J/(g*k)) um 120k
	Outputs	**Outputs**		
	Thermal pretreated maize silage	thermisch vorbehandelte Maissilage	145,69 kg	Hochrechnung aus Kleinversuchen
	Water vapour	Wasserdampf	24,31 kg	Berechnung uas Kleinversuchen
3 Vorhydrolyse Maissilage 55°C (prehedrolysis of maize silage 55°C)	**Inputs**	**Inputs**		
	Cellulase	Cellulase	5,2448 kg	Hochrechnung aus Kleinversuchen
	Electricity	Strom	14,394 MJ	6mal Rühren für 1h innerhalb 24h (Energieverbrauch für Rühren bei Maischversuchen gemessen)
	Thermal pretreated maize silage	thermisch vorbehandelte Maissilage	145,69 kg	
	Thermal energy	thermische Energie	6,0979 MJ	Nachheizen der Maische um 10°C innerhalb 24h
	Outputs	**Outputs**		
	Maize silage saccharified	Maissilagemaische verzuckert	145,69 kg	
4 Aufbereitung Triticale (conditioning of triticale)	**Inputs**	**Inputs**		
	Electricity	Strom	2,146 MJ	entnommen aus Studie Senn
	Thermal energy	thermische Energie	5,559 MJ	
	Triticale	Triticale	41,764 kg	
	Outputs	**Outputs**		
	Waste heat	Abwärme	9,903 MJ	
	Water vapour	Wasserdampf	0,97 kg	
	Triticale	Triticale	40,793 kg	
5 Vermahlung Triticale (milling of triticale)	**Inputs**	**Inputs**		
	Triticale	Triticale	1000 kg	
	Electricity	Strom	22 MJ	entnommen aus Studie Senn
	Outputs	**Outputs**		
	Milled Triticale	Triticale vermahlen	1000 kg	
6 Amylolytischer Aufschluß Maissilage und Triticale (2h) (amylolytic hydrolysis of maize silage and triticale)	**Inputs**	**Inputs**		
	GC 626	GC 626	$6{,}556 * 10^{-6}$ kg	Angabe Enzymhersteller
	Opimasch BG	Opimasch BG	$1{,}311 * 10^{-6}$ kg	Angabe Enzymhersteller
	Electricity	Strom	6,7221 MJ	Hochrechung aus Maischversuchen
	Thermal energy	thermische Energie	7,7186 MJ	
	Milled Triticale	Triticale vermahlen	40,793 kg	
	Outputs	**Outputs**		
	Saccharified mash	verzuckerte Maische	186,48 kg	
7 Fermentation (fermentation)	**Inputs**	**Inputs**		
	Saccharified mash	verzuckerte Maische	186,48 kg	
	Yeast	Hefe	0,79921 kg	
	GC 106	GC 106	$1{,}075 * 10^{-5}$ kg	Angabe Enzymhersteller
	Stargen 001	Stargen 001	0,0159 kg	Angabe Enzymhersteller
	Outputs	**Outputs**		
	Carbon dioxide	Kohlendioxid	17,281 kg	stöchiometrische Berechnung
	Fermented mash	vergorene Maische	169,2 kg	Berechnung
8 Destillation (distillation	**Inputs**	**Inputs**		
	Fermented mash	vergorene Maische	169,2 kg	
	Electricity	Strom	5,16 MJ	Studie Senn
	Thermal energy	thermische Energie	100,49 MJ	Studie Senn
	Outputs	**Outputs**		
	Ethanol	Ethanol	18,09 kg	Hochrechnung aus Maischversuchen
	Stillage	Schlempe	151,11 kg	Berechnung
9 Biogasprozess (biogas)	**Inputs**	**Inputs**		
	Stillage	Schlempe	151,11 kg	Berechnung
	Maize sillage 35%DS	Silomais 35%TS	41,758 kg	Berechnung
	Triticale straw 90%DS	Triticalestroh 90%TS	41,765 kg	Berechnung
	Outputs	**Outputs**		
	Biogas	Biogas	28,264 kg	Berechnung aus Biogasversuchen 14,615kg TS aus Schlempe [0,344m3 CH4/kg TS] und 34,674kg TS aus Triticalestroh [0,276 m3 CH4/kg TS]
	Electricity	Strom	149,67 MJ	30% Wirkungsgrad Quelle: Handreichung Biogas Model 1 Seite 207
	Thermal energy	thermische Energie	199,57 MJ	40% Wirkungsgrad Quelle: Handreichung Biogas Model 1 Seite 207

Distillery with fossil energy supply

Nummer im Flowchart/ Number in flow chart				
1 Silomais Hammermühle (maize silage hammermilled)	**Inputs**	**Inputs**		
	Maize silage 35%DS	Silomais 35%TS	100 kg	
	Water	Wasser	70 kg	gewogen
	Electricity	Strom	9 MJ	Leitfaden Bioenergie 25kWh/t
	Outputs	**Outputs**		
	Dispersed maize silage	Dispergierte Maissilage	170 kg	
2 Pretreatment Biomasse 160°C 30min (pretreatred biomass 160°C 30min)	**Inputs**	**Inputs**		
	Dispersed maize silage	Dispergierte Maissilage	170 kg	
	Thermal energy	thermische Energie	79,61 MJ	Berechnung: Erhitzen von 144,5 kg Wasser (4,1855J/(g*k)) und 25,5kg Stärke (2,3J/(g*k)) um 120k
	Outputs	**Outputs**		
	thermal pretreated maize silage	thermisch vorbehandelte Maissilage	145,69 kg	Hochrechnung aus Kleinversuchen
	Water vapour	Wasserdampf	24,31 kg	Berechnung uas Kleinversuchen
3 Vorhydrolyse Maissilage 55°C (prehydrolysis maize silage 55°C)	**Inputs**	**Inputs**		
	Cellulase	Cellulase	5,2448 kg	Hochrechnung aus Kleinversuchen
	Electricity	Strom	14,394 MJ	6mal Rühren für 1h innerhalb 24h (Energieverbrauch für Rühren bei Maischversuchen gemessen)
	thermal pretreated maize silage	thermisch vorbehandelte Maissilage	145,69 kg	
	Thermal energy	thermische Energie	6,0979 MJ	Nachheizen der Maische um 10°C innerhalb 24h
	Outputs	**Outputs**		
	Saccharified maize silage	Maissilagemaische verzuckert	145,69 kg	
4 Aufbereitung Triticale (conditioning of triticale)	**Inputs**	**Inputs**		
	Electricity	Strom	2,146 MJ	entnommen aus Studie Senn
	Thermal energy	thermische Energie	5,559 MJ	
	Triticale	Triticale	41,764 kg	
	Outputs	**Outputs**		
	Waste heat	Abwärme	9,903 MJ	
	Water vapour	Wasserdampf	0,97 kg	
	Triticale	Triticale	40,793 kg	
5 Vermahlung Triticale (milling of triticale)	**Inputs**	**Inputs**		
	Triticale	Triticale	1000 kg	
	Electricity	Strom	22 MJ	entnommen aus Studie Senn
	Outputs	**Outputs**		
	Milled triticale	Triticale vermahlen	1000 kg	
6 Amylolytischer Aufschluß Maissilage und Triticale (2h) (amylolytic hydrolysis of maize silage and triticale)	**Inputs**	**Inputs**		
	GC 626	GC 626	$6{,}556 * 10^{-6}$ kg	Angabe Enzymhersteller
	Opimasch BG	Opimasch BG	$1{,}311 * 10^{-6}$ kg	Angabe Enzymhersteller
	Electricity	Strom	6,7221 MJ	Hochrechung aus Maischversuchen
	Thermal energy	thermische Energie	7,7186 MJ	
	Milled triticale	Triticale vermahlen	40,793 kg	
	Outputs	**Outputs**		
	saccharified mash	verzuckerte Maische	186,48 kg	
7 Fermentation (fermentation)	**Inputs**	**Inputs**		
	Saccharified mash	verzuckerte Maische	186,48 kg	
	Yeast	Hefe	0,79921 kg	
	GC 106	GC 106	$1{,}075 * 10^{-5}$ kg	Angabe Enzymhersteller
	Stargen 001	Stargen 001	0,0159 kg	Angabe Enzymhersteller
	Outputs	**Outputs**		
	Carbon dioxide	Kohlendioxid	17,281 kg	stöchiometrische Berechnung
	Fermented mash	vergorene Maische	169,2 kg	Berechnung
8 Destillation (distillation)	**Inputs**	**Inputs**		
	Fermented mash	vergorene Maische	169,2 kg	
	Electricity	Strom	5,16 MJ	Studie Senn
	Thermal energy	thermische Energie	100,49 MJ	Studie Senn
	Outputs	**Outputs**		
	Ethanol	Ethanol	18,09 kg	Hochrechnung aus Maischversuchen
	Stillage	Schlempe	151,11 kg	Berechnung
9 Biogasprozess (biogas process)	**Inputs**	**Inputs**		
	Stillage	Schlempe	151,11 kg	Berechnung
	Maize silage 35%DS	Silomais 35%TS	41,758 kg	Berechnung
	Triticale straw 90%DS	Triticalestroh 90%TS	41,765 kg	Berechnung
	Outputs	**Outputs**		
	Biogas	Biogas	50,884 kg	Berechnung aus Biogasversuchen 14,615kg TS aus Schlempe [0,344m3 CH4/kg TS] und 34,674kg TS aus Triticalestroh [0,276 m3 CH4/kg TS]

Distillery with fossil energy supply and modern distillation using energy recovery

Nummer im Flowchart/Number in flow chart				
1 Silomais Hammermühle (maize silage hammermilled)	**Inputs**	**Inputs**		
	Maize silage 35%DS	Silomais 35%TS	100 kg	
	Water	Wasser	70 kg	gewogen
	Electricity	Strom	9 MJ	Leitfaden Bioenergie 25kWh/t
	Outputs	**Outputs**		
	Dispersed maize silage	Dispergierte Maissilage	170 kg	
2 Pretreatment Biomasse 160°C 30min (pretreatred biomass 160°C 30min)	**Inputs**	**Inputs**		
	Dispersed maize silage	Dispergierte Maissilage	170 kg	
	Thermal energy	thermische Energie	79,61 MJ	Berechnung: Erhitzen von 144,5 kg Wasser (4,1855J/(g*k)) und 25,5kg Stärke (2,3J/(g*k)) um 120k
	Outputs	**Outputs**		
	thermal pretreated maize silage	thermisch vorbehandelte Maissilage	145,69 kg	Hochrechnung aus Kleinversuchen
	Water vapour	Wasserdampf	24,31 kg	Berechnung uas Kleinversuchen
3 Vorhydrolyse Maissilage 55°C (prehydrolysis maize silage 55°C)	**Inputs**	**Inputs**		
	Cellulase	Cellulase	5,2448 kg	Hochrechnung aus Kleinversuchen
	Electricity	Strom	14,394 MJ	6mal Rühren für 1h innerhalb 24h (Energieverbrauch für Rühren bei Maischversuchen gemessen)
	thermal pretreated maize silage	thermisch vorbehandelte Maissilage	145,69 kg	
	Thermal energy	thermische Energie	6,0979 MJ	Nachheizen der Maische um 10°C innerhalb 24h
	Outputs	**Outputs**		
	Saccharified maize silage	Maissilagemaische verzuckert	145,69 kg	
4 Aufbereitung Triticale (conditioning of triticale)	**Inputs**	**Inputs**		
	Electricity	Strom	2,146 MJ	entnommen aus Studie Senn
	Thermal energy	thermische Energie	5,559 MJ	
	Triticale	Triticale	41,764 kg	
	Outputs	**Outputs**		
	Waste heat	Abwärme	9,903 MJ	
	Water vapour	Wasserdampf	0,97 kg	
	Triticale	Triticale	40,793 kg	
5 Vermahlung Triticale (milling of triticale)	**Inputs**	**Inputs**		
	Triticale	Triticale	1000 kg	
	Electricity	Strom	22 MJ	entnommen aus Studie Senn
	Outputs	**Outputs**		
	Milled triticale	Triticale vermahlen	1000 kg	
6 Amylolytischer Aufschluß Maissilage und Triticale (2h) (amylolytic hydrolysis of maize silage and triticale)	**Inputs**	**Inputs**		
	GC 626	GC 626	$6{,}556 * 10^{-6}$ kg	Angabe Enzymhersteller
	Opimasch BG	Opimasch BG	$1{,}311 * 10^{-6}$ kg	Angabe Enzymhersteller
	Electricity	Strom	6,7221 MJ	Hochrechung aus Maischversuchen
	Thermal energy	thermische Energie	7,7186 MJ	
	Milled triticale	Triticale vermahlen	40,793 kg	
	Outputs	**Outputs**		
	saccharified mash	verzuckerte Maische	186,48 kg	
7 Fermentation (fermentation)	**Inputs**	**Inputs**		
	Saccharified mash	verzuckerte Maische	186,48 kg	
	Yeast	Hefe	0,79921 kg	
	GC 106	GC 106	$1{,}075 * 10^{-6}$ kg	Angabe Enzymhersteller
	Stargen 001	Stargen 001	0,0159 kg	Angabe Enzymhersteller
	Outputs	**Outputs**		
	Carbon dioxide	Kohlendioxid	17,281 kg	stöchiometrische Berechnung
	Fermented mash	vergorene Maische	169,2 kg	Berechnung
8 Destillation (distillation)	**Inputs**	**Inputs**		
	Fermented mash	vergorene Maische	169,2 kg	
	Electricity	Strom	5,16 MJ	Studie Senn
	Thermal energy	thermische Energie	60,78 MJ	Alcohol Textbook Angabe Katzen
	Outputs	**Outputs**		
	Ethanol	Ethanol	18,09 kg	Hochrechnung aus Maischversuchen
	Stillage	Schlempe	151,11 kg	Berechnung
9 Biogasprozess (biogas process)	**Inputs**	**Inputs**		
	Stillage	Schlempe	151,11 kg	Berechnung
	Maize silage 35%DS	Silomais 35%TS	41,758 kg	Berechnung
	Triticale straw 90%DS	Triticalestroh 90%TS	41,765 kg	Berechnung
	Outputs	**Outputs**		
	Biogas	Biogas	50,884 kg	Berechnung aus Biogasversuchen 14,615kg TS aus Schlempe [0,344m3 CH4/kg TS] und 34,674kg TS aus Triticalestroh [0,276 m3 CH4/kg TS]

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