Master thesis

Production of ethanol and xylitol from hemicellulose hydrolysate

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ADH	Alcohol dehydrogenase
AR	L-arabinose reductase
C5 sugars	Pentose sugars
C6 sugars	Hexose sugars
CDM	Cell dry mass
demi. water	Demineralized water
EtOH	Ethanol
HMF	Hydroxymethylfurfural
HPLC	High pressure liquid chromatography
LAD	L-xylulose by L-arabitol-4-dehydrogenase
LXR	L-xylulose reductase
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide Adenine Dinucleotide plus Hydrogen
OD_{600}	Optical density measured at a wavelength of 600 nm
PDC	Pyruvate decarboxylase
PPP	Pentose phosphate pathway
Xall	Xylose and all other sugars (mannose, glucose, galactose, arabinose)
XDH	Xylitol dehydrogenase
XI	Xylose isomerase
XK	Xylulokinase
XR	D-xylose reductase

VII **Abstract**

In recent years, the saving of resources and efficient use of raw materials has gained strong interest. One of the big topics is the efficient utilization and bioconversion of lignocellulosic waste material from energy crops, wood and agricultural residues with microorganism, for the production of valuable chemicals. Lignocellulose is a complex biopolymer which can be broken down through pretreatment to a hemicellulose hydrolysate, containing pentose and hexose sugars.

The aim of this master thesis was to examine 6 pre-screened yeast strains (A027, C238, C240, C245, C255, HA1129) of the Vogelbusch Biocommodities GmbH cell bank on their ability to ferment the sugars in hemicellulose hydrolysate to produce either ethanol or xylitol with a high yield and a high production rate. The yeast strains were tested on synthetic media and on wheat straw hemicellulose hydrolysate. During the pretests, the yeast strains were tested in culture bottles and afterwards the best yeast strains were tested in fed-batch fermenter runs.

The tested yeast strains fully degraded the contained hexose sugars in 20-250 hours. The xylose was degraded slower than the hexoses (C6), while the arabinose was the least favored sugar. The fastest xylose degradation rates (0,17-0,34 g/L/h) were measured with strains C238 and C245 (*Candida sp.*).

The highest maximum ethanol concentration (16,5 g/L) was reached by the yeast strain C238 in the fermenter runs with hydrolysate-medium, but most of ethanol was formed from the C6 sugars (0,20-0,22 g ethanol/g hexose+ pentose sugars; 0,03-0,17 g ethanol/ g hexose sugars). The best ethanol yields were shown by the engineered *Saccharomyces cerevisiae* strain A027 in the synthetic medium (0,29 g ethanol/ g hexose+ pentose sugars; 0,25 g ethanol/g pentose sugars). In the hydrolysate-medium the fermentation and ethanol yields were high (0,26-0,27 g ethanol/ g hexose+ pentose sugars; 0,44-0,46 g ethanol/g pentose sugars), but the formation was inefficient, due to the poor xylose degradation rate (0,01 g/L/h). None of the strains in the experiments reached an ethanol yield or concentration high enough for an economical use.

The fermenter runs of *Candida* sp. C245 with synthetic medium showed a xylitol yield of 0,60-0,73 g/g and C238 a yield of 0,49-0,56 g/g. In the pretests, C245 showed a 20% lower and C238 about 50% higher yield than in the fermenter runs. In the fermenter runs with hydrolysate-medium, the xylitol yield for C238 was 28% higher (0,53-0,64 g/g) than for C245 (0,46 g/g).

In the pretests with hydrolysate-medium, the addition of peptone and yeast extract showed a 50% lower xylitol concentration and instead a 50% higher biomass formation for C245 (*Candida* sp.) and C255 (*Candida lignohabitans).*

2 Introduction & aim of work

In times of global warming and an imminent energy- and resource crisis, the saving of resources and efficient use of raw material has gained strong interest in recent years.

Biomass, produced by photo-synthesis, is a type of solar energy and therefore a promising option of renewable bioenergy (Sánchez and Cardona, 2008, Huang et al., 2011). Especially the utilization and bioconversion with microorganisms of lignocellulosic waste material from energy crops, wood and agricultural residues, plays a big role in the production of bioethanol, but also in the production of valuable chemicals like xylitol or 2,3-butanediol (Saha, 2003, Lin and Tanaka, 2006, Huang et al., 2011).

Bioethanol is recently produced from starchy and sugary biomass, the so called first generation feedstocks. These feedstocks have the disadvantage that they compete with human nutrition. Lignocellulosic biomass is abundant and therefore does not show this disadvantage (Walker, 2010).

According to Sánchez and Cardona (2008) the major potential of biomass feedstock in the world has rice straw, corn stover in the US and wheat straw in Europe. With regard to ethanol production, Kim and Dale (2004) estimated the global potential from lignocellulose feedstocks would be 491 GL/year. This amount of ethanol would replace 32% of the global gasoline consumption. The annually available amount of wheat straw accounts 430 Mt, which could be used for the production of 120 GL ethanol/year (Talebnia et al., 2010).

Lignocellulosic biomass consists of a complex structure of cellulose, hemicellulose and lignin. For the conversion into valuable substances, the complex structure of lignocellulosic biomass needs to be pretreated, to obtain a hemicellulose hydrolysate with hexose and pentose sugars (Sun and Cheng, 2002). During the pretreatment the formation of inhibitory compounds is possible (Palmqvist and Hahn-Hägerdal, 2000). The sugar and inhibitor composition of the hydrolysate depends on the used feedstock and the pretreatment method, nevertheless there is always a high amount of the pentose sugar xylose present (Chundawat et al., 2011). The efficient fermentation of hexoses, particularly glucose, by yeasts like *Saccharomyces cerevisiae* is already used in industrial scale, but the search for efficient xylose-fermenting microorganisms is still continuing. For an efficient fermentation of hemicellulose hydrolysate all sugars contained in the hydrolysate should be utilized (Talebnia et al., 2010) and the yeasts should be withstanding the inhibiting conditions of the hemicellulose hydrolysate-medium.

The aim of this master thesis was to examine pre-screened yeast strains of the Vogelbusch Biocommodities GmbH cell bank on their ability to ferment hemicellulose hydrolysate and produce either ethanol or xylitol with a high yield and at a high rate. The additionally purpose was a low by-product formation and biomass formation.

The yeast strains therefore were tested on a synthetic medium (Xall-medium) and hemicellulose hydrolysate-medium produced from wheat straw. The yeast strains should possibly fully utilize the contained hexose and pentose sugars of the medium. Also, the strains should be robust towards the hydrolysate conditions, like possible contained inhibitors. All yeast strains were tested in small culture bottles and the best yeast strains of the small-scale experiments were cultivated in the fermenter with a controlled air supply.

3 Background information

3.1 Lignocellulose biomass & hydrolysate

3.1.1 Structure of lignocellulosic biomass

The world biomass consists to 50% of the renewable, organic material called lignocellulosic biomass (Winkelhausen and Kuzmanova, 1998, Claassen et al., 1999). Estimated by Claassen et al. (1999) 10-50 billion tons lignocellulosic biomass are produced per year. Lignocellulosic materials are separated to six groups: crop residues, hardwood, softwood, cellulose wastes, herbaceous biomass and municipal solid wastes. The group with the greatest global potential are crop residues like rice straw, wheat straw, corn stover, cane bagasse, rice hulls or pulp (Sánchez and Cardona, 2008).

Lignocellulose is a biopolymer with a complex structure, consisting of the three main components: cellulose, hemicellulose and lignin. The major component of lignocellulose, with 40-60% of total dry weight, is cellulose. Cellulose is a homopolymer of β-1,4-linked D-glucose units. The linear cellulose chains are highly crystalline and tightly packed. This makes it challenging to break cellulose up, even with enzymes (Palmqvist and Hahn-Hägerdal, 2000, Talebnia et al., 2010, Madhavan et al., 2012).

20- 40% of plant biomass is composed of hemicelluloses (Hamelinck et al., 2005). Hemicelluloses are highly branched heteropolysaccharides, which are composed of the pentoses D-xylose and D-arabinose, the hexoses D-mannose, D-glucose, D-galactose and uronic acids (Palmqvist and Hahn-Hägerdal, 2000, Stambuk et al., 2008). The pentose and hexose monomers are linked via glycosidic bonds and acetylation (Stambuk et al., 2008). The dominant sugar type in the hemicelluloses, decides on the designation as mannan, xylan or galactan (Stambuk et al., 2008). Of those three, xylan is the most abundant.

The hemicellulose structure of softwoods, hardwoods and agricultural residues is different. Softwood hemicellulose has a higher amount of mannose and glucose units and only 10-12% pentose fraction. While the pentose fraction in hardwoods is between 19-33% and in agricultural residues up to 40%. For this reason, the fermentation of xylose is a bigger topic in the fermentation of agricultural residues and hardwood (Singh and Mishra, 1993, Chandrakant and Bisaria, 1998, Olofsson et al., 2008, Stambuk et al., 2008). Hemicellulose links the cellulose fibers with lignin and has a loose structure with low crystallinity. Hemicellulose can be therefore easier hydrolyzed than cellulose. (Talebnia et al., 2010, Madhavan et al., 2012)

Almost 10-30% of lignocellulose consists of lignin. Lignin is a complex three-dimensional aromatic network synthesized from phenylpropanoid precursors. Like an amorphous mass, lignin is surrounding the cellulose and hemicellulose fibers. Lignin has a strong influence on the breakdown of lignocellulosic biomass. A skeleton of phenylpropane units is connected to aromatic alcohol, like coniferyl alcohol, sinapyl alcohol, and p-coumaryl alcohol. Depending on the phenylpropanoid skeleton, called the lignin is either called “guaiacyl lignin” or “guaiacyl-syringyl lignin”(Palmqvist and Hahn-Hägerdal, 2000, Talebnia et al., 2010, Madhavan et al., 2012).

In this work, the hemicellulose hydrolysate was produced from wheat straw, which is typically composed of 33-40% (w/w) cellulose, 20-25% (w/w) hemicellulose, 15-20% (w/w) lignin, a small amount of soluble substrates and ash (Prasad et al., 2007, Talebnia et al., 2010). Xylan, with a D-xylose as backbone and different side groups, is the dominant hemicellulose polymer in wheat straw (Mazeau et al., 2005).

3.1.2 Pretreatment

The complex structure of the lignocellulosic materials is the reason why lignocellulosic biomass should be pretreated and saccharified or hydrolyzed, before it can be used for fermentation. A lot of pretreatment methods are available to break up the lignocellulosic matrix into cellulose, hemicellulose and lignin. The pretreatment also has the purpose to lower the crystallinity of the cellulose (Sánchez and Cardona, 2008). The pretreatment improves the following release of the monomeric hexose and pentose sugars from the cellulose and hemicellulose polysaccharides via hydrolysis (Sun and Cheng, 2002).

The pretreatment methods for lignocellulosic biomass can be divided into (Sánchez and Cardona, 2008, Talebnia et al., 2010):

- **Physical pretreatment:** Milling, grinding or chipping. Useful for size reduction of the particles and decreases the crystallinity of cellulose.
- **Physical-chemical pretreatment:** Steam explosion, Liquid hot water (LHW), Ammonia Fiber Explosion (AFEX)
- **Chemical pretreatment:** Concentrated- and/or diluted-acid hydrolysis with H_2SO_4 or HCl at high temperatures, Alkaline process with NaOH or lime, alkaline/oxidative pretreatment with peroxide or peracetic acid in combination with alkaline (e.g. NaOH), ozonolysis, Wet oxidation with water and high pressure oxygen/air at high temperature
- **Biological pretreatment:** selective degradation of lignin and hemicellulose with brown-, white-, and soft-rot fungi for example with *Pleurotus ostreatus*, *Aspergillus niger* or *Aspergillus awamori*

These methods can help to increase the surface area, the size of the pores or the porosity. The physical-chemical, chemical and biological methods also help to degrade the hemicellulose and to transform/degrade the lignin. Depending on the feedstock a different pretreatment method is useful. Usually the methods are combined to improve the effect of the process, for example a combination of a mechanical method together with thermal and chemical effects (Talebnia et al., 2010).

Besides of the fermentable sugars, inhibitory compounds can be formed of the lignocellulosic biomass, depending on the process conditions of the pretreatment and the raw material (Olofsson et al., 2008, Madhavan et al., 2012). The main inhibitors are furfural, 5-hydroxymethyl furfural (HMF), acetate, formic acid and several phenolic compounds (Palmqvist and Hahn-Hägerdal, 2000). Furfural originates from the further degraded xylose of the hemicellulose, while HMF is formed by the degradation of the hexose sugars mannose, galactose and glucose (Palmqvist and Hahn-Hägerdal, 2000). High temperatures and pressure have an influence on the inhibitor formation. The deacetylation of hemicellulose can cause the formation of acetic acid, while the breakdown of furfural and HMF can form formic acid (Dunlop, 1948, Ulbricht et al., 1984). Several phenolic compounds like

e.g. p-hydroxybenzaldehyde and cinnamaldehyde, are generated by the degradation of lignin (Pérez et al., 2002).

The inhibitory effect of the furans, furfural and HMF, mostly affect the growth of the yeast cells and therefore also decrease the productivity of the fermentation (Madhavan et al., 2012). More toxic than furfural and HMF are the lignin degradation products (Parajó et al., 1998b). Acetic acid inhibition depends on the pH in the fermentation broth, because of the increase of undissociated molecules at lower pH, which can diffuse across the plasma membrane of the yeast cells. The acid molecules dissociate in the neutral cell cytosol, reduce the intracellular pH, leading to impaired ion transport and therefore increased energy requirement (Palmqvist and Hahn-Hägerdal, 2000, Madhavan et al., 2012).

The yeasts strains can be either selected to tolerate the inhibitory compounds or the hydrolysate is detoxified to lower the inhibitors concentration and improve the fermentability. There are different detoxification methods, the common methods are: overliming with $Ca(OH)_2$, enzymatic treatment with laccases and soft-rot fungi, adsorption on wood charcoal, ion-exchange resin or zeolites (Madhavan et al., 2012).

The appropriate pretreatment method is chosen depending on the factors of high sugar release efficiency, low inhibitor formation, low energy consumption, minimum use of chemicals, need of recovery of catalysts or recycling of solvents and operating expenses (Talebnia et al., 2010, Huang et al., 2011). The current pretreatment methods for the breakdown of the lignocellulose matrix are complicated, energy-consuming and account for 33% of the total process costs (Tomás-Pejó et al., 2008). Consequently, the methods are still in the development stage (Sánchez and Cardona, 2008).

For the pretreatment in this work, the wheat straw was physically pretreated with a mill at the beginning. Afterwards concentrated acid-hydrolysis with hydrochloric acid at room temperature and diluted-acid hydrolysis at high temperatures was implemented (more information about development and optimization of the used pretreatment can be read at the master thesis of Meyer (2017)).

The concentrated acid-hydrolysis has the advantage, that subsequently no saccharification with enzymes is needed. The drawbacks, beside of high consumption of acid and energy, are the corrosion of the equipment, long time reaction and the required acid recovery (Galbe and Zacchi, 2002, Sun and Cheng, 2002). In the diluted acid-hydrolysis, the high temperature attains an acceptable conversion of cellulose to glucose. A problem is posed by the non-selectivity and therefore decomposition of hemicellulose sugars and increased corrosion of the equipment. The greatest problems is the formation of many inhibiting by-products and the requirement of pH neutralization for the usage as fermentation medium (Talebnia et al., 2010).

3.1.3 Hydrolysis

The next step after the pretreatment is the hydrolysis of the polysaccharides into fermentable sugars. There are two ways for hydrolyzing the β 1-4 glycosidic bonds linking the glucose units of cellulose: acid hydrolysis or enzymatic hydrolysis (Helle and Duff, 2004).

For acid hydrolysis, the pretreated lignocellulose is processed with concentrated acid at low temperatures, which results in high glucose yields. The drawbacks of this hydrolysis method is the

possible formation of inhibitory compounds, the difficult recovery and the corrosive properties of the concentrated acid. (Helle and Duff, 2004)

The most effective method is therefore the enzymatic hydrolysis, where the sugar molecules of the cellulose matrix are liberated with three groups of cellulases: endoglucanases, exoglucanases and β-glucosidase (Talebnia et al., 2010). The enzymes work together. The endoglucanases attack the cellulose randomly at regions with low crystallinity, while the exoglucanases can detach cellobiose units from the now exposed ends of the cellulose chains. The cellobiose is cleaved into glucose monomers by the β-glucosidase (Helle and Duff, 2004, Talebnia et al., 2010).

The main problem of the enzymatic hydrolysis is to achieve acceptable conversion yields, while the problem of end-product inhibition, of exo-and endo-glucanase by cellobiose and β-glucosidase by glucose, can occur. The required high enzyme loading levels take in the biggest part of the operation costs. (Helle and Duff, 2004, Talebnia et al., 2010)

The composition of the finished hemicellulose hydrolysate depends on the used lignocellulosic feedstock, the pretreatment method and the chosen hydrolyzation (Chundawat et al., 2011). Nigam (2001) reported a composition of wheat straw hydrolysate with 12,8 g/L xylose, 1,7 g/L glucose and 2,6 g/L arabinose, established by sulfuric acid hydrolysis and detoxification. While Canilha et al. (2006) achieved a composition of 15,4 g/L xylose, 4,4 g/L glucose, 2,2 g/L arabinose, 1,2 g/L acetic acid, 0,53 g/L furfural and 0,09 g/L HMF by diluted sulfuric acid hydrolysis.

3.2 Ethanol

The usage of ethanol (C_2H_6O, ethyl alcohol, bioethanol) has a long history. The first combustion engines 1826 were already able to use ethanol as fuel (Demirbas et al., 2009). At the beginning of the 20th century bioethanol was widely used as fuel in Europe and the USA, till the first world war made the production uneconomical compared to fuels of mineral oil. The usage was growing again, when Brazil introduced a pioneer project and the oil crisis 1970 lead to a recovery of the economical market (Solomon et al., 2007, Balat and Balat, 2009, Demirbas et al., 2009). Especially the customization of the car engines, which allow to switch between ethanol and gasoline, made it possible that bioethanol is today the most important biofuel around the world. The global ethanol production was 65,4 billion liters in 2008 (Mussatto et al., 2010) and in 2015 only the global fuel ethanol production was 97,3 billion liters (= 25,6 billion US gallons (Renewable Fuels Association, 2016)). 73% of the produced ethanol is used as fuel ethanol, 17% as beverage ethanol and 10% is industrial ethanol (Sánchez and Cardona, 2008). The leading producers of the worldwide production are the US with 57% and Brazil with 28% (Renewable Fuels Association, 2016).

With the usage of bioethanol as fuel, the fossil fuels can be substituted and the emission of carbon dioxide can be reduced due to the cleaner combustion. The high octane value, good combustion efficiency and the advantages that it is non-toxic and does not contaminate water sources, bioethanol can be also used as oxygenate instead of MTBE (Methyl tert-butyl ether) to raise the octane number (Sánchez and Cardona, 2008, Madhavan et al., 2012). Therefore, petrol is blended with bioethanol. On this way the burning of petrol is made more efficient and completely, which helps to reduce emissions (Madhavan et al., 2012).

Many countries passed laws for the addition of biofuels, like bioethanol, to gasoline. In Austria, the share of biofuels among fuels was regulated to be 5,75% starting October 2008 (Rechtsinformationssystem, 2012), while in Germany the directive 2009/30/EG of the EU Parliament obliges the offer of the fuel "E10". E10 contains beside of the gasoline 10% v/v ethanol.

Currently the production of ethanol is based on sugar and starch based materials e. g. sugarcane and grains, the so called "first generation" feedstocks (Sánchez and Cardona, 2008, Talebnia et al., 2010). On this way bioethanol competes with human food production by using valuable arable land. The increasing food prices lead to the "food or fuel" discussion (Waskow and Burdick, 2009). The additional estimated rising feedstock prices of the current used feedstocks like corn, enhanced the interest in lignocellulose biomass as feedstock for bioethanol production (Talebnia et al., 2010). Lignocellulosic biomass, the second generation feedstocks, are abundant and cannot be used for food production (Walker, 2010). The main challenge is the efficient utilization of the hemicellulose sugars (hexose and pentose, but especially xylose) of the lignocellulosic feedstocks by suitable yeasts, which could reduce the cost of ethanol production by 25% (Madhavan et al., 2012).

To be competitive with fossil fuel production costs, it is estimated by Walker (2010) that the ethanol price should be maximum 0,2 €/liter. To achieve this price on industrial scale, the fermentation broth must contain more than 4% (w/w) ethanol, to have an economically recovery of the ethanol by distillation (Zhang et al., 2010). The converted ethanol concentration should be therefore approximately 42 g/L.

3.3 Xylitol

Xylitol ($C_5H_{12}O_5$) is a pentahydroxy sugar alcohol with a sweetening power similar to sucrose. In nature it can be found in fruits and vegetables, it also occurs as intermediate in the mammalian carbohydrate metabolism. The production in the average adult human is between 5- 15 g xylitol/day (Winkelhausen and Kuzmanova, 1998).

Xylitol has similar properties like sucrose: same sweet, caloric content of 17 kJ/kg and dissolves readily in water. The sweetening power of xylitol is interesting, because of its advantages of a slow adsorption without the distribution of insulin and limited fluctuation of the blood glucose levels and is therefore recommended for diabetic patients (Parajó et al., 1998a, Winkelhausen and Kuzmanova, 1998). Another advantage of xylitol is that it inhibits the growth of oral bacteria and reduces the formation of dental caries. Regarding to caries prevention, xylitol is considered to be the best of all alternative sweeteners (Parajó et al., 1998a, Winkelhausen and Kuzmanova, 1998). As food ingredient it has several advantages for example of not undergoing a Maillard reaction or giving a feeling of vaporization in the oral and nasal cavities, because of the negative heat of dissolution (Parajó et al., 1998a).

For some mammals, for example dogs, xylitol is toxic (Peterson, 2013). In the human body xylitol can only cause secondary gastrointestinal effects, like harmless osmotic diarrhea. The maximum consumption per day is therefore limited to 20 g, but an adaptation to increased amount is possible (Parajó et al., 1998a, Winkelhausen and Kuzmanova, 1998).

Due to the numerous advantages xylitol creates great interest in the food industry, but the high production costs (about 10 times that of sucrose or sorbitol) of the current methods limit the market to use this sweetener (Winkelhausen and Kuzmanova, 1998). Xylitol can be produced through solid-liquid extraction from fruits or vegetables, but due to low concentrations in raw material and the high cost this method is very uneconomical. The current used method on large-scale is the chemical reduction of xylose, which is derived from wood hydrolysates. The main steps are: acid hydrolysis of plant material, purification of hydrolysate to receive pure xylose, hydrogenation of the xylose to xylitol and crystallization of the xylitol. The disadvantages in this process are the low yield of 50-60% obtained of the initial xylose concentration and the expensive required purification and separation steps of the xylose and xylitol. (Parajó et al., 1998a, Winkelhausen and Kuzmanova, 1998)

To overcome the drawbacks of the current methods, the most attractive procedure is the microbial production. The microbial production of xylitol was always associated with the ethanol production of D-xylose with yeasts, but only as a by-product. When the interest in xylitol as alternative sweetener grew, the possibility of xylose fermentation for the production of xylitol received more attention (Winkelhausen and Kuzmanova, 1998).

3.4 Pentose uptake & metabolism in yeasts

The fermentation of hexose sugars by yeasts is generally well studied. *Saccharomyces cerevisiae* is the model organism on which the yeast metabolism was explored. Due to the fact of the inability of *S. cerevisiae* to ferment pentose sugars (Hahn-Hägerdal et al., 1994), the metabolic pathways of pentose sugars were investigated on the basis of native xylose-fermenting yeasts like *Candida shehatae* or *Pichia stipites*.

In this chapter the sugar transport, metabolic pathway and importance of oxygen for the fermentation of pentoses and hexoses of hemicellulose hydrolysate are described.

3.4.1 Transport systems

The transport of the pentose sugars across the plasma membrane into the cell cytoplasm of the yeasts is the first and significant rate limiting step of the pentose fermentation (Madhavan et al., 2012).

The sugar uptake in yeasts can happen by facilitated diffusion and/or by active transport mechanisms. While the facilitated diffusion happens spontaneous without energy requirement, the active transport requires metabolic energy (e.g. H^+ symport system needs the proton motive force) for the uptake, but can also work against concentration gradients (McMillan, 1993).

Although *S. cerevisiae* cannot metabolize xylose, it can still take it up by facilitated transport through hexose transporters (Olofsson et al., 2008). However, the affinity for the uptake is 200-fold lower than for glucose and glucose competitively inhibits the xylose assimilation (Lee et al., 2002, Olofsson et al., 2008, Stambuk et al., 2008).

Native xylose and/or arabinose fermenting yeast species often have a high capacity, low affinity facilitated diffusion system and a low capacity, high affinity arabinose/ xylose H^+ symport system. The low affinity facilitated diffusion is shared by glucose and xylose, while the high affinity is specific for

pentose sugars (Leandro et al., 2006, Madhavan et al., 2012). Even though the transport covers a wide range of sugar concentrations, the affinities for xylose are lower than for glucose (Stambuk et al., 2008).

A sequential pattern for the utilization of the sugars was determined, which could be affected by the sugar uptake. When the medium contains D-glucose, D-mannose or D-galactose beside of D-xylose, the xylose is not utilized until the glucose is fully consumed, but the xylose degradation begins, when there are still some of the hexoses present in the medium (Lee et al., 1996, Winkelhausen and Kuzmanova, 1998).

3.4.2 Pentose sugar metabolism

After the uptake of the D-xylose into the yeast cell it is converted to D-xylulose. While bacteria can convert D-xylose directly with the enzyme xylose isomerase (XI, xylA pathway, see Figure 1 no. 4), yeasts convert the D-xylose in a two-step reduction and oxidation (Jeffries, 2006). The first step is the conversion of D-xylose to D-xylitol by the NAD(P)H-dependent D-xylose reductase (XR). Xylitol is either assimilated or oxidized to D-xylulose in the second step, by NAD^+-dependent D-xylitol dehydrogenase (XDH) (Hahn-Hägerdal et al., 1994).

The L-arabinose goes through an analog pathway, by being converted by L-arabinose reductase (AR) into the polyol L-arabitol and the oxidation to L-xylulose by L-arabitol-4-dehydrogenase (LAD). Instead of channeling the L-xylulose into the xylose-pathway, the L-xylulose is converted into D-xylitol by L-xylulose reductase (LXR). Via the xylose pathway the D-xylitol is converted into D-xylulose (Kordowska-Wiater, 2015).

The formed D-xylulose is subsequent phosphorylated by the D-xylulokinase into D-xylulose 5-phosphate, which is afterwards metabolized in the pentose phosphate pathway (PPP). The PPP provides via an oxidative and non-oxidative phase: NADH, D-ribose for the nucleic acid biosynthesis and erythrose 4-phosphate which the cell needs for the syntheses of aromatic amino acids, but also CO_2 (Hahn-Hägerdal et al., 1994, Jeffries, 2006). All pentoses have to be shuttled through the PPP. In contrast, in the hexose metabolism only approximately 0,9-10% of glucose in form of fructose-6-phosphate are going into the PPP and the other part is metabolized via the glycolysis (Hahn-Hägerdal et al., 1994).

Depending on the amount of fructose-6-P cycled in the PPP or metabolized by glycolysis, the ethanol yield from xylose is different. When all fructose-6-P goes into the glycolysis the yield is around 0,51 g/g, while the yield is around 0,31 g/g, when it is cycled in the PPP (Hahn-Hägerdal et al., 1994). A reason presumed by Slinger et al. (1990), could be the high level of carbon dioxide, produced in the PPP of some xylose-fermenting yeasts.

In the final fermentation steps, the metabolites of the PPP (fructose 6P and glyceraldehyde 3P) are metabolized to pyruvate by glycolysis. The pyruvate is converted to ethanol via pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH). NADH of the oxidation of glyceraldehyde-3-P is reoxidized (Hahn-Hägerdal et al., 1994).

The metabolism of the pentose and hexose sugars, occurring in hemicellulose hydrolysate, is outlined in Figure 1.

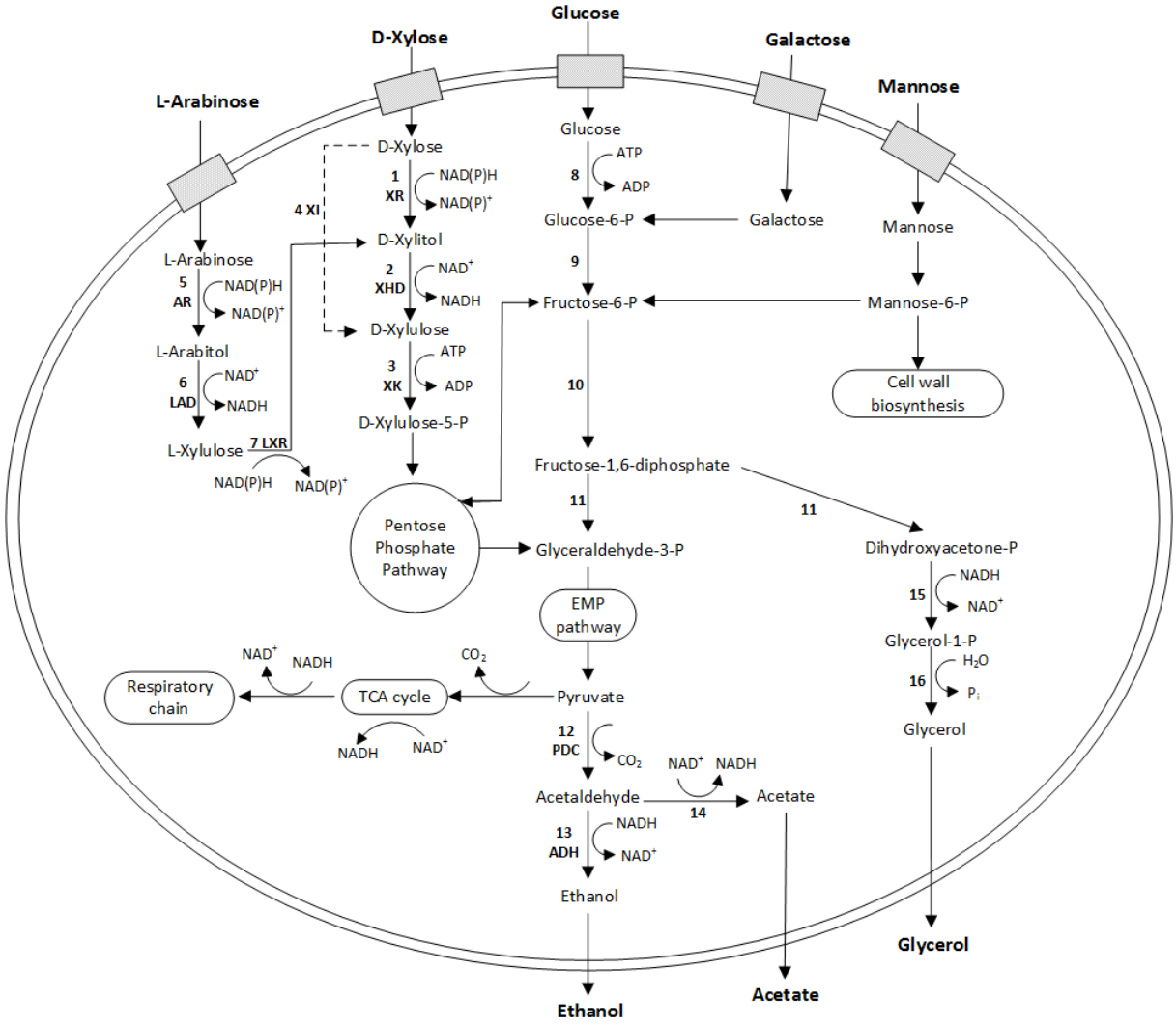

Figure 1: Sugar metabolism of the main hemicellulose hydrolysate pentose and hexoses in yeasts. 1 Xylose reductase (XR), 2 Xylitol dehydrogenase (XHD), 3 Xylulokinase (XK), 4 Xylose isomerase (XI), 5 L-arabinose reductase (AR), 6 L-arabitol-4-dehydrogenase (LAD), 7 L-Xylulose reductase (LXR), 8 Hexokinase, 9 Phosphoglucose isomerase, 10 Phosphofructokinase, 11 Aldolase, 12 Pyruvate decarboxylase (PDC), 13 Alcohol dehydrogenase (ADH), 14 Acetaldehyde dehydrogenase, 15 Glycerol phosphate dehydrogenase, 16 Glycerol phosphatase; EMP pathway = Embden-Meyerhof-Parnas pathway (multiple steps); TCA cycle = tricarboxylic acid cycle. The dotted arrow represents the xylA pathway present in bacteria and some fungi. (Hahn-Hägerdal et al., 1994, Helle and Duff, 2004, Stambuk et al., 2008, Madhavan et al., 2012)

3.4.3 The importance of the oxygen supply

When oxygen is present, the pyruvate is oxidized through the tricarboxylic acid (TCA) cycle and the respiratory chain. Xylose-fermenting yeasts like *Pichia stipitis*, *Pachysolen tannophilus* and *Candida shehatae* are proven to require oxygen for the growth on xylose (Hahn-Hägerdal et al., 1994). According to Laplace et al. (1991) excess oxygen supply increases the cell mass, because of the deviation of the pyruvate from the fermentative pathway to the TCA cycle, and therefore lowers the ethanol yield.

Additionally, several studies with different *P. stipitis* and *C. shehatae strains,* proven that the rate of transport was limited under aerobic and oxygen limited conditions and thus also the utilization of D-xylose is limited (Hahn-Hägerdal et al., 1994, Winkelhausen and Kuzmanova, 1998). Under anaerobic conditions the transport was not limited, but instead the two-initial steps of xylose metabolism were affected by the absence of oxygen (Winkelhausen and Kuzmanova, 1998).

The main reason for the importance of the oxygen regulation, is the shift from ethanol production to xylitol accumulation, which is attributed to the redox imbalance of the cofactors of the xylose reduction by XR and xylitol oxidation by XDH (Novy et al., 2014). The conversion of xylitol into xylulose by xylitol dehydrogenase (XDH) is the rate-limiting step of the ethanol production and also the key for a high yield in xylitol accumulation (Parajó et al., 1998a).

The cofactor imbalance occurs in yeasts with a NADPH-dependent XR and NAD^+-dependent XDH. In the absence of oxygen or other electron acceptor these yeasts can still maintain NADPH, but are incapable to regenerate NAD^+. The inactivity of XDH leads to the accumulation of the intermediate xylitol (Parajó et al., 1998a, Madhavan et al., 2012). Xylitol is not produced under fully aerobic conditions (Winkelhausen and Kuzmanova, 1998). When xylitol production is the goal, a very low amount of oxygen, individually for each yeast strain, should be supplied (Winkelhausen and Kuzmanova, 1998).

Yeasts with a NADH-dependent XR and NAD^+-dependent XDH, would theoretically produce ethanol under anaerobic conditions, but are not able to grow on D-xylose without any oxygen. According to Kuriyama and Kobayashi (1993) oxygen limitation is the best way to maintain fermentation and simultaneously growth.

Yeasts which have XR with a dual cofactor demand of NADH and NADPH, can regenerate NAD^+. In this yeasts the predominant product under anaerobic and oxygen-limited condition is ethanol (Parajó et al., 1998a). Many types of *P. stipites*, *C. shehatae* and *P. tannophilus* do have this property of a dual cofactor specificity, according to Hahn-Hägerdal (1994). This author recommended oxygen limited conditions for a high ethanol production.

It can be noted, that the L-arabinose metabolism shows the same phenomenon of cofactor imbalance for the L-arabinose reductase (AR) and L arabitol-4-dehydrogenas (LAD). This means, that arabitol is accumulated under low oxygen conditions (Kordowska-Wiater, 2015).

3.5 Suitable yeasts & cultivation conditions

The suitable yeast for the fermentation of hemicellulose hydrolysate should fulfill several requirements (Hahn-Hägerdal et al., 2007, Olofsson et al., 2008, Madhavan et al., 2012): high ethanol or xylitol yield, high productivity, low by-product formation, tolerance to inhibitors present in the hydrolysate, ability to utilize all sugars present in the hydrolysate, withstand high osmotic pressure and yeasts for ethanol production should withstand high ethanol concentrations for lower distillation costs.

In this chapter, we will have a look the current known yeasts, which are suitable to produce ethanol or xylitol of pentose and hexose sugars.

3.5.1 Ethanol production

The work horse and best known microorganism for ethanol production from hexoses is the yeast *Saccharomyces cerevisiae*. This yeast shows a high ethanol yield (at optimal conditions higher than 0,45 g/g), a high specific rate (up to 1,3 g/g/h) and is proven to be safe and efficient for large scale production, because of the high ethanol tolerance with over 100 g/L and robustness against other inhibitors (Olofsson et al., 2008, Talebnia et al., 2010, Madhavan et al., 2012).

The major disadvantage of this yeast strain is the inability to utilize the pentose sugars xylose and arabinose, which are typical sugars in hemicellulose hydrolysate (Talebnia et al., 2010). To solve these problems, native *S. cerevisiae* were genetically modified by the introduction of bacterial genes encoding xylose isomerase (XI) (for the direct conversion of D-xylose to D-xylulose) or genes for xylose reductase (XR) and xylitol dehydrogenase (XDH) from fungi (Olofsson et al., 2008). In addition, the genes of xylulokinase (XK) needs to be overexpressed and transport proteins are needed for efficient uptake and fermentation of xylose (Olofsson et al., 2008). Despite of all these modifications, the engineered *S. cerevisiae* still has a low ethanol productivity and additionally the implemented plasmids with the new genes are often rejected by the host cells (Krishnan et al., 2000, Watanabe et al., 2007).

Another option for the production of ethanol from hemicellulose hydrolysate, is the usage of naturally xylose-fermenting yeasts. Many studies showed the suitability of pentose fermentation of: *Candida shehatae, Pichia stipitis*, *Pichia segobiensis, Pachysolen tannophilus, Hansenula sp., Debaromyces sp., Schwanniomyces sp.* and many new xylose-fermenting species were isolated for example from beetles (Jeffries, 2006, Stambuk et al., 2008, Talebnia et al., 2010).

The currently best xylose-fermenting yeast strains for ethanol production are *P. stipitis* and *C. shehatae*, with a proven maximum specific rate up to 0,51 g/g/h and an ethanol yield up to 0,50 g/g (Hahn-Hägerdal et al., 1994).

These yeasts also have considerable drawbacks, such as the 2-4 times lower tolerance to ethanol (Claassen et al., 1999) and low tolerance towards inhibitor compounds of the undetoxified hydrolysate (Olofsson et al., 2008, Stambuk et al., 2008). Also it was described, that the specific ethanol productivity with xylose-fermenting yeasts was fivefold lower, than that obtained by fermentation of glucose with *S. cerevisiae* (Hahn-Hägerdal et al., 1994). For example, *C. shehatae* with 4.4 g/L/h compared to *S. cerevisiae* with 170 g/L/h (Olsson and Hahn-Hägerdal, 1996).

Like described in chapter 3.4.3, the oxygen supply must be really low and always controlled, to maintain an efficient production of ethanol from xylose (Olofsson et al., 2008). Oxygen is therefore one of the most important parameters in the ethanol production. The optimal temperature for the efficient ethanol production is, according to Talebnia et al. (2010), between 30-35°C, while some studies show that some yeast strains are able to grow between 0-48°C (Ho and Powel, 2014).

Dependent on the yeast strain, the optimal pH for growth is between 4 and 6. In case of big difference of the intracellular pH of the cells and the media (e.g. through a high acetic acid concentration in the media (Madhavan et al., 2012)), the yeast cells spend energy to maintain a constant intracellular pH-value. In the worst case, this can lead to a stop of the cell metabolism and therefore stops the growth (Narendranath and Power, 2005).

3.5.2 Xylitol production

Yeasts are considered to be the best microorganisms for xylitol production. The best yeast strains reported by Winkelhausen and Kuzmanova (1998) were of the *Candida* genus, like *C. guilliermondii*, *C. mogii* and *C. tropicalis*. With *Candida guilliermondii* FTI 20037 , a xylitol concentration of 30,8 g/L with a xylitol yield of 0,88 g/g and a productivity of 0,57 g/L/h was attained from wheat straw hydrolysate by Canilha et al. (2006). In contrast, according to Winkelhausen and Kuzmanova (1998) the xylitol production of *C. guilliermondii* FTI 20037 from rice straw had a yield of 0,69 g/g and from sugar cane bagasse 0,48 g/g. The high xylitol yield of 0,88 g/g was also reported for *Candida* sp. 11-2 from sugar cane bagasse (Winkelhausen and Kuzmanova, 1998).

Parajó et al. (1998a) pointed out the importance of a high XR or low XDH activity as a criterion for xylitol-producing yeast strains, which can be found for example in *Candida pelliculosa (Kitpreechavanich et al., 1984)*.

The ideal conditions for the xylitol production from xylose are very differently, depending on the used yeast strain. As earlier described (see chapter 3.4.3), the oxygen supply is one of the main control parameters for xylitol production. The second important influence factor is the xylose concentration, because of the activation of xylose reductase (XR) and xylitol dehydrogenase (XDH) by D-xylose. By increasing the xylose concentration, the xylitol formation is favored over ethanol, which leads to an increased xylitol yield. The highest xylitol yields for the most yeasts are claimed to be achieved with an initial xylose concentrations between 100-200 g/L. (Winkelhausen and Kuzmanova, 1998)

Winkelhausen and Kuzmanova (1998) also determined, that the most suitable temperature for xylitol production seems to be 30°C, while small temperature variations in the range of 30-37°C do not really affect the xylitol production. Higher temperatures around 36°C also seem to increase the xylitol formation (du Preez et al., 1986). The optimum initial pH for a fermentation without pH-control, seems to be pH 7, while it was shown that the pH-optimum for cultivation and xylitol formation can be between pH 2,5-6 (Winkelhausen and Kuzmanova, 1998).

For some yeast strains a higher initial cell concentration appears to have a positive effect on the xylitol production from xylose. Also different media composition obtained an increase of the xylitol yield, like the supplementation of yeast extract, urea, biotin or methanol (Winkelhausen and Kuzmanova, 1998).

4 Material and methods

4.1 Yeast strains

In the previous screenings 91 different yeast strains of the Vogelbusch Biocommodities cell bank were tested. In this work, only the 6 most promising yeast strains of the preliminary tests were investigated.

Among the best yeast strains were yeasts with the prefixes A for “Alcohol yeast”, C for “Fodder yeast” and HA for “pre-screened strains which can metabolize pentoses (C5 sugars)”.

Table 1 gives an overview over all yeast strains that were used in this study. A027, C238, C255 and HA1129 were selected due to the high ethanol-levels in preliminary testing. C245 and C240 had generated the highest xylitol-concentrations.

Table 1: Selected yeast strains of the pre-screened strains

designation	*strain*
A027	*Saccharomyces cerevisiae*
C238	unknown
C240	*Candida utilis*
C245	*Candida* sp.
C255	*Candida lignohabitans*
HA1129	*Candida intermedia (ATCC 201070)*

Saccharomyces cerevisiae strains are naturally not able to ferment xylose (Hahn-Hägerdal et al., 1994). The yeast strain A027 (in other references it is named BP10001) is a genetic engineered *S. cerevisiae* strain with an inserted NADH-preferring xylose reductase (XR) of *Candida tenuis*. On this way A027 should be able to ferment xylose and form ethanol with an increased yield (Novy et al., 2014).

C238 is a fodder yeast, which is yet an unknown strain.

4.2 Experimental design

The experiments were split up in two parts: the pre-experiments, which were carried out in culture bottles, and the main experiments in small scale fermenters. Both experiment-parts needed a pre-culture for the inoculation. The experimental procedures are described in this chapter.

All used equipment is listed in chapter 9.2. The equipment which came into contact with the yeasts, was sterilized either by dry heat in a drying oven at 180 °C for at least 5 h or with steam in an autoclave at 121 °C and 2 bar for 20 min. Some materials were bought packaged individually and sterile. Steps which required sterile conditions were performed in the laminar air flow work bench.

4.2.1 Preparation of pre-cultures

All yeast strains were organized in Master and Working Cell Banks (MCB, WCB) which were stored in cryo vials (5 mL sterile, Roth) at -80 °C.

For inoculation, a cryo vial of the desired WCB was thawed and the pre-culture was prepared for each strain. For the experiments in the culture bottles 100 mL YPG-medium with 1 mL of WCB was poured into a sterile 250 mL Erlenmeyer flask which was sealed with a cotton plug.

For the fermenter pre-cultures two sterile 300 mL Erlenmeyer flasks with cotton plugs were prepared, filled with 100 mL of YPG-medium and 1 mL of WCB was pipetted into. The pre-cultures were incubated on a shaker overnight (160 rpm, 30 °C, 24 h).

4.2.2 Fermentation in culture bottles

4.2.2.1 Culture bottle setup

The pre-experiments were performed in culture bottles (200 mL, Duran). Each bottle contained a magnetic stirring bar (30x6 mm, VWR). For sampling a tube was added (silicone, peroxide tubing 2x4 mm, VWR) with a clamp on the end of the tube to ensure sterility. Before each bottle was sealed with a cotton stopper, 20 µL of 1: 10 diluted antifoam solution were filled into each bottle. The assembled culture bottles were autoclaved and aseptically filled with 100 mL of the particular medium (see chapter 4.3).

Figure 2: Culture bottles setup in the incubator. Negative control Xall-medium A and inoculated with strain C238 (right).

4.2.2.2 Culture approach in the culture bottles

24 hours after inoculation the starter cultures should have been in the exponential growth phase and were taken from the shaker. For each strain 50 mL of the cell culture were harvested and filled into sterile centrifuge tubes. To wash out the remaining ingredients of the YPG-medium the cultures were centrifuged at 4000 rpm for 7 min, the pellets were resuspended in 50 mL of 0,9 % (w/v) NaCl solution and centrifuged again. Afterwards each cell pellet was resuspended in 15 mL medium. The optical density of the inoculum was measured at a wavelength of 600 nm (OD_{600}) by diluting each cell culture with demi. water to get a value between 0,2 and 0,6. All fermentations were started with an OD_{600} value of 0,5 which could be achieved by calculating the inoculation volume that would be necessary for a specific turbidity. The inoculation volume was calculated by the following equation:

$$V_{\text{inoculum}}[\text{mL}] = \frac{OD_{600}\ (\text{desired}) * V_{\text{culture medium}}[\text{mL}]}{OD_{600}\ (\text{inoculum})}$$

Formula 1: Calculation of inoculum volume of the culture bottles

For double determination two culture bottles were inoculated from one starter culture with the same inoculation volume at the same time. One culture bottle for every kind of medium was left without inoculum as the negative control. Also the sample for the zero-time point (T0) was taken of the pure medium.

After inoculation, the bottles were additionally sealed with a plastic paraffin film (“Parafilm”) to minimize the air exchange. Before incubating the culture bottles, the first sample was taken (time point T1), like described in chapter 1.5. The bottles were placed on stirring plates in the incubator with 32 °C and 150 rpm.

4.2.3 Fermentation in 1L fermenter with micro-aeration

4.2.3.1 Fermenter setup

The main fermentation experiments took place in glass fermenters with a filling volume of 1 liter.

Many studies (see chapter 3.4) and previous tests of Vogelbusch indicated that the yeasts are unable to grow on xylose in a strictly anaerobic environment. To find a balance between a good xylose degradation and a high ethanol or xylitol concentration, micro-aeration was applied on the fermenter-experiments.

Figure 3: Fermenter setup - on the right the glass fermenter on the magnetic stirrer, on the left the pump which pushes the air into fermenter

The micro-aeration of the fermenter culture was achieved with a peristaltic pump. The pump sucks in the ambient air over a tube and pushes it through a sterile filter and a glass frit into the fermenter. The pump was operated with 20 rpm, so the aeration in the 1L fermenter was about 0,004 vvm. The measurement of the entering air is described in chapter 4.2.3.3. For sterile air outlet, a fermentation lock was assembled in the lid of the fermenter.

For sterile sampling a glass tube was plugged into the lid of the fermenter and a rubber hose with a clamp was put over the end of the glass tube to ensure sterile sample taking.

To ensure good mixture a magnetic stirrer (Janke & Kunkel, KMO2) on agitation speed 3 was used. The temperature control for 32 °C, was done with a water bath connected to the double jacket of the fermenter.

The assembled fermenter was filled with 60 mL demineralized water and 200µL 1:10 diluted antifoam agent. For the last step of the assembling the fermenter was autoclaved.

4.2.3.2 Filling and inoculation of the fermenter

The medium (preparation see chapter 4.3) was filled into the fermenter and was mixed. Afterwards 20 mL medium for the zero sample (2,5 mL) and the resuspension of the inoculum cell pellet was taken out of the fermenter.

Two times 50 mL pre-culture broth were harvested from the overnight pre-culture. The samples were centrifuged at 4.000 rpm for 7 min in sterile centrifuge tubes. The supernatant was discarded, each pellet was resuspended in 50 mL 0,9% NaCl-solution and again centrifuged (4.000 rpm, 7 min). Afterwards both pellets were resuspended in a total volume of 10 mL medium.

The optical density of the inoculum was measured as described for the culture bottles in chapter 4.2.2.2. The inoculation volume for the fermenter was calculated by previous equation:

$$V_{\text{inoculum}}[\text{mL}] = \frac{OD_{600}\ (\text{desired}) * V_{\text{fermenter}}[\text{mL}]}{OD_{600}\ (\text{inoculum})}$$

Formula 2: Calculation of inoculum volume of the fermenter

The volume in the fermenter was calculated as follows:

$$V_{fermenter} = 1000mL - 2{,}5mL - 10mL + volume\ of\ the\ inoculum$$

The last step was the inoculation with the calculated volume. Unused medium was given back into the fermenter. The fermenter was incubated with an agitation speed 3 of the magnetic stirrer, at a temperature of 32 °C and a micro-aeration of approx. 0,004 vvm.

4.2.3.3 Examination of micro-aeration rate in the fermenter

To measure the air-input by the peristaltic pump, the fermenter was set up like in the real experiments but filled with one liter water only. A silicon tube was connected with the outside top of the fermentation lock. Next to the fermenter a glass beaker was filled with water and a water filled measuring cylinder was dipped reverse, with the glass opening, into it. Now the tube of the fermenter was put into the upside-down measuring cylinder.

The peristaltic pump was started and after a few minutes the air bubbled out of the fermenter continuously, since the pressure was equalized. The aeration rate was determined by measuring the water volume displaced by the air coming out of the fermenter in a certain time.

The displaced air was measured at 20 rpm and 40 rpm of the feeding pump with the same peristaltic pump tube size. The average air flow was 11 mL air per minute (0,011 vvm) with 40 rpm and 4 mL air per minute (0,004 vvm) with 20 rpm. The entire measurement results can be looked up in appendix chapter 9.3.

4.2.3.4 Fed-batch Fermenter

In the fermenter-experiments as soon as the xylose in the fermenter had been degraded to a certain limit more xylose was added to the fermenter to increase the xylose concentration again. Fermenters with synthetic medium were fed with 100 g xylose and 10 g arabinose. The fermenters which contained hydrolysate-medium were fed with 100 g xylose and, depending on the concentration ratios of the hydrolysate, glucose and arabinose. The sugars were dissolved in 100 mL or 150 mL demi. water. The solution was autoclaved.

Before the new substrates were added to the fermenter, a sample was taken for the measurement of the current optical density, HPLC, pH and dry matter. Subsequently the sugar-solutions were poured with a sterile funnel into the large opening of the fermenter, whereas the aeration was adjusted on the highest rate to decrease the entering of contaminations. The fermenter content was stirred on a high agitation speed for at least 15 min. Then another sample was taken and optical density, HPLC and pH were measured again.

4.3 Media

For the pre-cultivation and cultivation of the yeasts, three different media were used. The preparation of the media is described in the following chapters.

4.3.1 Preparation of YPG-medium

For the pre-cultures a simple medium with yeast extract, peptone and glucose was used. The concentrations of the components are listed in Table 2:

Table 2: Composition of pre-culture YPG-medium

Substances	Concentrations
Tryptone/peptone	20 g/L
Yeast extract	10 g/L
D-Glucose	10 g/L

The peptone and yeast extract solution was autoclaved (CertoClave, Classic 125°C/140°C) separate from the glucose solution to prevent a Maillard reaction.

4.3.2 Preparation of synthetic Xall-medium

The Xall-medium that was used for the fermentations, should be similar to a sugar composition of a hemicellulose hydrolysate. Table 3 gives an overview of the composition of the Xall-medium.

The medium was chemically defined and contained 30 g/L xylose as well as 3 g/L of glucose, galactose, mannose and arabinose.

To find out if peptone and yeast extract enhance the fermentation, those components were added to the Xall-medium. The basic Xall-medium is called “A”. The medium with peptone and yeast extract is called Xall-medium “B” (see Table 4).

Table 3: Composition of basic Xall-medium A

Components	Concentrations
Total sugar concentration	42 g/L
Xylose	30 g/L
Arabinose	3 g/L
Glucose	3 g/L
Galactose	3 g/L
Mannose	3 g/L
Nutrient salts	
$(NH_4)_2HPO_4$	3 g/L
K_2HPO_4	15 g/L
$CaCl_2.2H_2O$	0,1 g/L
Vitamin solution	1,23 mL/L
Trace elements solution 1	4,94 mL/L
H_2SO_4 (100 g/L) to adjust pH to 6,00	approx. 40 mL/L

Table 4: Composition of Xall-medium B, basic Xall with peptone and yeast extract

Components	Concentrations
Total sugar concentration	42 g/L
Xylose	30 g/L
Arabinose	3 g/L
Glucose	3 g/L
Galactose	3 g/L
Mannose	3 g/L
Nutrient salts	
$(NH_4)_2HPO_4$	3 g/L
K_2HPO_4	15 g/L
$CaCl_2.2H_2O$	0,1 g/L
Peptone	1 g/L
Yeast Extract	0,5 g/L
Vitamin solution	1,23 mL/L
Trace elements solution 1	4,94 mL/L
H_2SO_4 (100 g/L) to adjust pH to 6,00	approx. 40 mL/L

The sugars, nutrient salts, $CaCl_2.2H_2O$ and for "Xall B" the peptone-yeast extract were weighed (concentrations see Table 3 and Table 4) and solved in separate flasks. To adjust the pH of the medium to pH 6, approximately 40 mL diluted H_2SO_4 per liter medium (concentration: 100 g/L) were added to the nutrient salt solution. The solutions were autoclaved (121 °C, 20 min, 2 bar) separately.

In the laminar flow work bench (Gelaire, Air Flow Class 100) the solutions were mixed together and the water loss was compensated with sterile demi. water. Then the sterile vitamin solution (preparation described in chapter 4.3.2.1) and the trace elements solution (preparation described in chapter 4.3.2.2) were added to the medium. The medium was stirred before use.

4.3.2.1 Vitamin solution

50 mL vitamin solution were prepared according to the concentrations in Table 5. The solution was divided into 1,5 mL portions and frozen at -20 °C until use. Before use, the vitamin solution was thawed, vortexed until the solution was clear again and filtered through a sterile syringe filter (Rotilabo, CA, 0,2 µm pore size, Ø 25 mm, Lactan).

Table 5: Composition of vitamin solution

Vitamins	Concentration	Amount for 50 mL
D-Biotin	0,05 g/L	0,0025 g
Calcium-D-pantothenate	2,60 g/L	0,1300 g
meso-Inositol	80,00 g/L	4,0000 g
Thiamine hydrochloride	1,60 g/L	0,0800 g
Pyridoxol hydrochloride	1,60 g/L	0,0800 g
4-Aminobezoic acid	0,80 g/L	0,0400 g
Nicotinic acid	1,60 g/L	0,0800 g

4.3.2.2 Trace elements solution

$MgSO_4.7H_2O$ was weighed separately from the other salts (for composition and concentrations see Table 6). The salts were diluted in a small amount of demi. water. After the two salt solutions prepared, they were combined in a 200 mL volumetric flask and 17 mL of H_2SO_4 (100 g/L) were added. For further dissolving the flask was left in an ultrasonic bath (Bandelin, Sonorex Transitor) for 10 minutes. In the end the solution was filled up to the 200 mL mark. The solution was stored in the refrigerator at 1,5 °C and was mixed before use. In the sterile bench the required amount was filtered through a sterile syringe filter (Rotilabo, CA, 0,2 µm pore size, Ø 25 mm, Lactan) and collected in a sterile 50 mL-SCHOTT-flask.

Table 6: Composition of trace elements solution

Trace elements	Concentration	Amount for 200 mL
$MgSO_4.7H_2O$	100 g/L	20,000 g
NaCl	12,5 g/L	2,500 g
$ZnSO_4.7H_2O$	4,5 g/L	0,900 g
$FeSO_4.7H_2O$	2,5 g/L	0,500 g
$CuSO_4.5H_2O$	0,16 g/L	0,032 g
$MnSO_4.H_2O$	0,27 g/L	0,054 g
$CoCl_2.6H_2O$	0,8 g/L	0,016 g
H_2SO_4 (100 g/L)	85 mL/L	17 mL

4.3.3 Production of straw hemicellulose hydrolysate

The process for the acid hydrolysis of straw hemicellulose was based on a technology described in "Chemical and biochemical generation of carbohydrates from lignocellulose-feedstock (*Lupinus nootkatensis*) - quantification of glucose" of Kamm et al. (2006). The process was adapted for our purpose. The process is explained in detail in the master thesis of Meyer (2017)

In the first steps of the hydrolysis wheat straw was milled in a household mixer (Commercial Blender, Waring) and afterwards finely ground (Retsch, SR2, mesh sieve 0,75 mm) to get a straw powder. 125 g wheat straw was weight into a 3 L glass flask. For the acid treatment 625 mL hydrochloric acid (32% [w/w]) were added and the mass was well mixed with a glass rod. The flask was sealed with a rubber plug and left for 24 hours at room temperature (app. 20°C).

The next day the flask was put into a boiling water bath and 1875 mL boiling demi. water was added. The solution was cooked for exactly 30 minutes and stirred from time to time.

To remove undissolved solids, the hydrolysate was filtrated (Büchner funnel with a perforated plate; circular filter paper Macherey-Nagel, MN640w, d=18,5 cm; vacuum flask; water jet pump). The filtration residue was washed three times each with 500 mL of demi. water.

The clear hydrolysate-solution was concentrated in the Rotavapor (parts: Büchi R-205, Vacuum controller Büchi V-800, heating bath Büchi B-490) at a maximum temperature of 40°C, a pressure of 40-50 mbar and a rotation speed of 280 rpm until it turned to a viscous syrup. The syrup was washed three times with 100 mL of demi. water.

The last steps of the process were to determine the total volume and the ingredients/ sugar concentration of the concentrated hydrolysate. For the determination of the sugar concentration a sample of 100 µL was taken and diluted by 9,9 mL of demi. water (1:100 dilution). The diluted sample was neutralized with NaOH (concentration 200 g/L) to reach a pH-value of 6. Afterwards the solution was filtrated (Chromafil Xtra CA-20/25, GF/C, 0,2 µm, Macherey-Nagel) into HPLC vials (Rotilabo, sample vials 2 mL, Roth Lactan) and measured on HPLC 1 and HPLC 2. The total sugar concentration in the hydrolysate-syrup and the yield of the acid treatment were calculated.

4.3.4 Preparation of hydrolysate-medium

The hemicellulose-hydrolysate was produced by acidic degradation of straw (see chapter 4.3.3).

For the hydrolysate-medium the solutions were prepared like the Xall-medium only the sugar-solution was replaced by the hydrolysate. There was also a version without ("A") and a version with ("B") peptone and yeast extract (see Table 7 and Table 8).

Table 7: Composition of hydrolysate-medium "A"

Components	Concentrations
Nutrient salts	
$(NH_4)_2HPO_4$	3 g/L
K_2HPO_4	15 g/L
$CaCl_2.2H_2O$	0,1 g/L
Vitamin solution	1,23 mL/L
Trace elements solution 1	4,94 mL/L
H_2SO_4 (100 g/L) to adjust pH to 6,00	approx. 40 mL/L

Table 8: Composition of hydrolysate-medium "B"

Components	Concentrations
Nutrient salts	
$(NH_4)_2HPO_4$	3 g/L
K_2HPO_4	15 g/L
$CaCl_2.2H_2O$	0,1 g/L
Peptone	1 g/L
Yeast Extract	0,5 g/L
Vitamin solution	1,23 mL/L
Trace elements solution 1	4,94 mL/L
H_2SO_4 (100 g/L) to adjust pH to 6,00	approx. 40 mL/L

The required amount of hydrolysate was calculated via the sugar-concentrations in the hydrolysate which were determined by the HPLC. The volume of hydrolysate was adjusted to a concentration of 30 g/L Xylose in the medium. When adjusted to 30 g/L Xylose the other contents in the hydrolysate were about: 7-10 g/L glucose, 4-5 g/L arabinose and 0,5-0,8 g/L acetate.

The hydrolysate was neutralized with NaOH (solution had a concentration of 200 g/L). The neutralized hydrolysate was transferred in sterile centrifuge vessels and was centrifuged (centrifuge:

Sigma, 6-10) at 8.000 rpm for 15 minutes to remove precipitates which formed during the neutralization.

The following steps were carried out in the laminar air flow work bench (Gelaire, Air Flow Class 100). The volume of the supernatant was measured with a sterile measuring cylinder. The hydrolysate was transferred into the medium. The volume difference to the total needed medium was calculated and the volume was compensated with sterile demi. water.

For the optical density measurement, the diluted medium was used as blank and was subtracted of the absorption of the sample-absorption.

4.4 Analytical methods

4.4.1 Sampling the culture bottles

For sampling the culture bottle was placed on a magnetic stirrer. A sterile syringe (Inject Single-Use syringes 2 mL, Braun) was put over the tip of the sampling-tube. The sample was drawn at the same time when the clamp was opened to prevent a backflow of unsterile liquid or air into the bottle. To rinse the tube 1,5 mL sample was discarded, afterwards the 2,5 mL sample were taken.

4.4.2 Sampling the fermenters

The stirring rate of the fermenter was increased for about 15 min, before the sample was taken. To prevent the water in the fermentation lock from being sucked in, while the sample was taken at the sampling-tube (which produces a vacuum), the pump for the air-input was set to maximum rpm. The sample was taken with a sterile syringe (Inject Single-Use syringes 2 mL, Braun) and the sample was drawn at the same moment when the clamp was opened to prevent a backflow of unsterile liquid or air. To rinse the tube 5 mL waste were drawn. After that 5 mL sample was drawn.

Figure 4: Lid of a fermenter with the fermentation lock in the middle, sampling tube with clamp on the left and tube for the air input on the

4.4.3 Optical density measurement

For measuring the optical density (OD) the samples were diluted to get an absorption value between 0,2 and 0,6. The samples were measured with a photometer at a wavelength of 600 nm, with demi. water as blank. Turbidity which was caused by medium components or antifoam solution, had to be determined from the time point zero measurement in the beginning of the experiment. The resulting value was multiplied with the dilution factor to get the actual optical density of the sample.

The relative standard deviation of the respective OD is given in Table 9.

Table 9: Relative standard deviation [%] of the optical density (OD) measurement (Vogelbusch internal laboratory data)

Optical density []	**1**	**2,5**	**5**	**10**	**20**
Rel. standard deviation [%]	4,01	3,51	2,39	4,63	7,52

4.4.4 pH measurement

A pH electrode (Schott H61) was used for the measurement of the pH-value. The calibration of the electrode was done every day, using pH 7 and pH 4 calibration puffer solutions (Merck).

4.4.5 High performance liquid chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) was used for the quantifying analysis of the sugars and metabolic products in the samples.

For the preparation of the HPLC analysis, the samples were centrifuged (3300 rpm, 1 min) and the supernatant of each sample was diluted 1:5 with demi. water (1440 µL demi. water and 360 µL sample). The diluted samples were pressed through a syringe filter (Chromafil Xtra CA-20/25, GF/C, 0,2 µm, Macherey-Nagel) to remove particles bigger than 0,2 µm and simultaneously transfer the sample into vials suitable for HPLC (Rotilabo sample vials 2 mL, Roth Lactan).

HPLC analysis was carried out by the HPLC units 1 and 2. The core of HPLC unit 1 is the organic acid analysis column. This column was used for the quantification of ethanol, acetic acid and glycerol. HPLC unit 2 was operated with carbohydrates analysis columns. Therefore, HPLC 2 was used to quantify xylose, arabinose, galactose, glucose, mannose, xylitol and arabitol/mannitol. Working with this method, arabitol and mannitol have the same retention times on HPLC2 column and could therefore not be separated. All information about the components and the different methods of HPLC1 and 2 are summarized in Table 10 and Table 11.

Table 10: Components of HPLC-units 1 and 2

	HPLC 1	HPLC 2
Column task	Organic acid analysis column	Carbohydrates analysis columns
Column(s)	**BioRad Aminex HPX-87H** Ion exclusion column 300*7,8 mm	**Phenomenex Rezex RPM-Monosaccharide** Ion exclusion Pb+2 (8%) column 300*7,8 mm
Pre-column	BioRad Micro Guard: Cation H-Cartridge 30*4,6 mm	BioRad Micro Guard: Carbo-P Cartridge 30*4,6 mm
Ion-exchange columns	none	**Cation exchange column:** Biorad De-Ashing Cartridge 20*4,6 mm **Anion exchange column:** Biorad De-Ashing Cartrige 20*4,6 mm
Communication module	Shimadzu CBM-20A	Shimadzu CBM-20A
Refractive index detector	Shimadzu RID-10A	Shimadzu RID-10A
Column oven	Shimadzu CTO-10AC	Shimadzu CTO-6A
Pump	Shimadzu LC-10AD	Shimadzu LC-20AD
Degasser	Shimadzu DGU-20A3	Shimadzu DGU-20A3
Auto sampler	Shimadzu SIL-20A HT	Shimadzu SIL-20A HT
Software	LC-Solution	LC-Solution

For the one-point calibration of the HPLC 1 and HPLC 2, two different external standards were used depending on the measured substances (see Table 12 and Table 13). The concentrations were located

between 0,5 and 8 g/L depending on the expected concentrations of the substances in the samples. The standards were measured three times and the mean results were used for the calibration.

Based on the calibration curves the HPLC software automatically calculated the concentrations of the substances from the particular peak areas in [g/L]. As a consequence of the 5-folded dilution of the HPLC samples, concentrations measured below 0,2 g/L were sometimes inaccurate.

According to the internal laboratory data Vogelbusch Biocommodities GmbH, the HPLC analysis showed in the range of 0,2 g/L up to high concentrations a deviation of lower than 1%.

Table 11: HPLC methods

	HPLC 1	HPLC 2
Eluent	0,0025 mol/L H_2SO_4	Ultrapure water
Flow rate	0,5 mL/min	0,9 mL/min
Injection volume	20 µL	6 µL
RI detector cell temperature	40 °C	40 °C
Column oven temperature	60 °C	85 °C
Analysis time	30 min	27 min

Table 12: Concentrations of the substances in the standard of HPLC 1 and their retention time

Flow rate: 0,5 mL/min	HPLC 1 [HPX-87H]	
	Retention time [min]	**Standard substances [g/100 mL]**
Glucose	11,35	0,20
Xylose	12,18	0,80
Arabinose	13,28	0,20
Xylitol	14,20	0,20
Glycerol	16,79	0,20
Acetate	18,64	0,2 (NaAc 0,2779g)
Ethanol	26,67	0,15

Table 13: Concentrations of the substances in the standard of HPLC 2 and their retention time

Flow rate: 0,9 mL/min	HPLC 2 [RPM Pb]	
	Retention time [min]	**Standard substances [g/100 mL]**
Glucose	10,26	0,20
Xylose	10,93	0,80
Galactose	11,46	0,15
Arabinose	12,17	0,20
Mannose	12,85	0,05
Ethanol	13,15	0,15
Arabitol/Mannitol	18,19	each 0,1
Xylitol	22,31	0,40

4.4.6 Analysis of inhibitors in the hydrolysate

The hydrolysates were analyzed for inhibitory substances such as furfural, hydroxymethylfurfural (HMF) and acetate. Samples were analyzed on HPLC 1 unit with a method called "Xylose-Inhibitors" (for parameters see Table 14). The hydrolysate was prepared by neutralization with sodium hydroxide (NaOH, conc. 200 g/L) and filtration into HPLC vials (as described in chapter 4.4.5).

The analysis results revealed no peaks for the retention time of furfural or HMF (see chromatograms in appendix 9.4). Acetate was present in the hydrolysate in small quantity, but was highly diluted in the hydrolysate-medium. On this account, it can be said that there are no significant inhibitor-concentrations in the hydrolysate or the final fermentation medium.

Table 14: Parameters of the "Xylose-Inhibitor" method of HPLC1

	HPLC 1
Eluent	0,0025 mol/L H_2SO_4
Flow rate	0,5 mL/min
Injection volume	20 µL
RI detector cell temperature	40 °C
Column oven temperature	60 °C
Analysis time	60 min

4.4.7 Determination of cell dry mass (CDM)

After the last sampling, the culture bottles or fermenters were opened and the cell dry mass (CDM) was determined from the remaining culture volume. The cell dry mass of the culture bottles was determined only one time. The cell dry mass determination for the fermenter runs was done in threefold determination. Glass microfiber filter disks (cut-off 0.20 µm, Ø 47 mm, Whatman, GE Healthcare) were placed in an aluminum tray and dried in a compartment dryer at 105 °C for at least 5 hours. The trays with the filters were cooled down in a desiccator. Afterwards they were weighed with an analytical balance to determine the TARA-weight. The culture bottles were placed on a magnetic stirrer while 5 mL sample was drawn. One filter was placed on a frit of a filter flask and the sample was applied and filtrated by applying vacuum to the filter flask with a water jet pump. The retained cells were washed 3 times with 5 mL demi. water to remove the rest of the culture medium. The tray was placed in the compartment dryer again and dried at 105 °C over night (or at least 5 hours). On the next day, the tray with the filter was transferred into a desiccator again and weighed out when it was cooled down.

The cell dry mass could be calculated by Formula 3:

$$\mathrm{CDM\ [g/L]} = \frac{m_f - m_{TARA}}{V_s \times 1000}$$

m_f … final weight of filter [g]
m_{TARA} … initial weight of filter [g]
V_s … Volume of sample used [mL]

Formula 3: Calculation of Cell Dry Mass [g/L]

The relative standard deviation [%] of the respective CDM [g/L] is given in Table 15 (source: Vogelbusch Biocommodities internal laboratory data).

Table 15: Relative standard deviation [%] of the cell dry mass (CDM) analysis

concentration [g/L]	1	2,5	5	10	20
Rel. standard deviation [%]	4,80	3,02	0,70	0,47	1,41

4.4.8 Microscopic control

Once a week the samples were examined under a microscope to assess the conditions of the yeast cells and for microbial contaminations. For this the cells were observed at 400x and 600x optical magnification.

4.4.9 Data Evaluation

4.4.9.1 CDM-OD_{600}-Ratio

The optical density (OD_{600}) was measured daily, while the cell dry mass (CDM) was determined only once at the end of the experiment. For the determination of the CDM over the runtime of the process, the correlation of CDM and OD_{600} was calculated.

$$CDM\ [\frac{g}{L * OD}] = \frac{CDM_{End}\ [\frac{g}{L}]}{OD_{600,End}[\ ]}$$

Formula 4: Calculation of the CDM-OD_{600}-Ratio [g/L/OD]

4.4.9.2 Ethanol Yield (Y_{EtOH})

Maximum theoretical ethanol yield from the C6 sugars (glucose, galactose and mannose) is defined as (Madhavan et al., 2012):

$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$ (without biomass formation)

$$maximum\ theoretical\ Y_{EtOH} = 0{,}51\ \frac{g\ EtOH}{g\ C6\ sugar}$$

The maximum ethanol yield was calculated by the measured maximum ethanol concentration and the difference of total sugar concentration from start till end.

$$max.Y_{EtOH} = \frac{max.ethanol\ [\frac{g}{L}]}{\Delta\ sugars\ at\ max.ethanol\ [\frac{g}{L}]}$$

Formula 5: Calculation of maximum ethanol yield

The formula was adjusted for the ethanol yield of the C5 and C6 sugars at the time point 0 g/L C6 sugars and for the ethanol yield of C5 sugars between the time point 0 g/L C6 sugars and the maximum ethanol concentration:

$$Y_{EtOH} = \frac{ethanol\ conc.\ at\ time\ point\ 0\ g/L\ C6\ [\frac{g}{L}]}{\Delta\ sugars\ at\ time\ point\ 0\ g/L\ C6\ \ [\frac{g}{L}]}$$

Formula 6: Calculation of ethanol yield from all sugars at time point 0 g/L C6 sugars

$$Y_{EtOH} = \frac{ethanol\ conc.\ at\ time\ point\ 0\ g/L\ C6\ [\frac{g}{L}]}{\Delta\ C6\ sugars\ at\ time\ point\ 0\ g/L\ C6\ \ [\frac{g}{L}]}$$

Formula 7: Calculation of ethanol yield from C6 sugars at time point 0 g/L C6 sugars

$$Y_{EtOH} = \frac{(maximum\ ethanol) - (ethanol\ conc.\ at\ time\ point \frac{0\ g}{L} C6)\ \ [\frac{g}{L}]}{(\Delta\ C5\ sugars\ at\ maximum\ ethanol) - (\Delta\ C5\ sugars\ at\ time\ point \frac{0\ g}{L} C6)\ \ [\frac{g}{L}]}$$

Formula 8: Calculation of ethanol yield from C5 sugars between time point 0 g/L C6 sugars and maximum ethanol concentration

4.4.9.3 Xylitol Yield ($Y_{Xylitol}$)

The maximum theoretical yield of xylitol from xylose is computed by Barbosa et al. (1988):

$$maximal\ theoretical\ Y_{Xylitol} = \ 0{,}9\ \frac{\text{g xylitol}}{\text{g xylose}}$$

Formula 9: Calculation of maximum theoretical xylitol yield from xylose (M: molar mass)

The yield of xylitol was calculated by assuming that all xylitol is derived from xylose. The yield was calculated by the measured maximum xylitol concentration and the difference of xylose concentration from start till end.

$$max.\ Y_{Xylitol} = \frac{max.\ xylitol\ [\frac{g}{L}]}{\Delta\ xylose\ at\ max.\ xylitol\ [\frac{g}{L}]}$$

Formula 10: Calculation of maximum xylitol yield

4.4.9.4 Biomass Yield

The biomass yield or CDM yield was calculated from the determined cell dry mass (CDM, measurement procedure see chapter 4.4.7) at the end of the experiments and the total sugar consumption (measured by HPLC, see chapter 4.4.5). The biomass production from other media compounds, like yeast extract and peptone, were neglected.

$$CDM\ yield = \frac{CDM\ [\frac{g}{L}]}{\Delta\ sugars\ at\ end\ [\frac{g}{L}]}$$

Formula 11: Calculation of biomass yield or CDM yield at the end of fermentation

The biomass yield was also calculated based on the C5 sugars:

$$CDM\ yield\ of\ C5\ sugars = \frac{\left(CDM\ at \frac{0\ g}{L} C6\right) - (CDM\ at\ end)\ [\frac{g}{L}]}{\left(\Delta\ C5\ sugars\ at \frac{0\ g}{L} C6\right) - (\Delta\ C5\ sugars\ at\ end)\ [\frac{g}{L}]}$$

Formula 12: Calculation of CDM yield with C5 sugars between 0 g/L C6 sugars and the end of fermentation

5 Results

The 6 most promising yeast strains of the preliminary tests (see chapter 4.1) were used for further testing in culture bottles and in small scale fermenters. The results are shown in the following chapters. The range of variation of the analysis methods is described in chapter 4.4 (Relative standard deviation for the OD: 2,39-7,52%; HPLC: < 1%; CDM: 0,47-4,80%).

5.1 Pretests in the culture bottles

In the first part of the experiments, the pre-selected strains were tested in small scale culture bottles with 250 ml volume (set-up see chapter 4.2.2).

Each test was carried out in double determination. In the first week of fermentation a sample from the bottles was taken each day. In the second and following weeks, samples were taken every second working day (see chapter 4.4.1). The samples were analyzed for optical density (OD_{600}, see chapter 4.4.3), pH-value (chapter 4.4.3) and the concentration of sugars and metabolic products by HPLC (chapter 4.4.5). Once a week the samples were microscopically examined (chapter 4.4.8). The tests were run until the xylose was completely metabolized. When the experiments were stopped the cell dry mass (CDM, see chapter 4.4.7) was determined.

5.1.1 Culture bottles with Xall-medium A & B

The tests were done with synthetic Xall-medium A (see chapter 4.3.2), which has a sugar composition similar to hemicellulose-hydrolysate substrates from wheat straw. For the optimization of the fermentation, the addition of peptone and yeast extract was tested. Xall-medium with supplements was called Xall-medium B (see chapter 4.3.2).

In the following tables the mean values of results from the duplicate testing of the strains in Xall-medium A are listed together with the results of Xall-medium B. The experiments were run until the xylose was fully degraded or until 500 hours. For this reason, the duration of the experiments is given in the tables. For better comparability of the results from different experiments the data from 305 hours are shown in the tables.

An overview of the **sugar consumption of the strains** in the culture bottles is shown in Table 16. The C6 sugars glucose, galactose and mannose were degraded completely in minimum 20 hours (yeast strains C238 and C240) and maximum 250 hours (yeast strain C255) in all tests.

The fastest **xylose degradation** was achieved by the yeast strains C238, C245 and C240 in Xall A. Under these conditions the strains degraded more than 95 % of the xylose in 305 hours. C240 in Xall B degraded about 84% of the xylose. The strains A027 and C255 were slower and degraded in the same time only 67-76 % of the available xylose.

The yeast strain C238 had fully degraded the xylose in both Xall-media within 477 hours. Xylose was degraded completely by A027 in Xall B by 497 hours, C240 in Xall A at 353 hours and C255 in Xall A at 497 hours. Whereas A027 in Xall A (98 % at 497 hours), C240 in Xall B (91% at 353 hours), C245 in both media (98-99% at 353 hours) and C255 in Xall B (99 % at 497 h) degraded nearly all xylose.

The strain HA1129 degraded less than 40 % of the xylose of Xall A & B in 503 h, which was the longest fermentation time and hence the slowest degradation of all yeast strains.

Table 16: Selected yeast strains in the culture bottles with Xall-medium A and B. Overview of the percentage of consumed sugar at 305 hours and at the end of the experiment.

Yeast strain	Run duration [h]	Medium	Δ Xylose 305h [%]	Δ Xylose End [%]	Δ Arabinose 305h [%]	Δ Arabinose End [%]	Δ 100% C6 sugars at __ hours
A027	497	Xall A	66,7	98,4	51,8	83,2	102,0
		Xall B	75,6	100,0	56,8	90,0	138,0
C238	476,5	Xall A	98,9	100,0	30,6	39,3	44,0
		Xall B	100,0	100,0	41,3	60,6	20,0
C240	353	Xall A	97,5	100,0	8,9	13,7	41,0
		Xall B	84,4	90,9	8,5	10,9	41,0
C245	353	Xall A	95,8	98,3	28,9	32,5	185,0
		Xall B	96,8	99,0	17,4	23,3	161,0
C255	497	Xall A	71,5	100,0	26,0	68,4	234,0
		Xall B	73,8	99,3	22,3	71,9	234,0
HA1129	503,25	Xall A	28,5	38,0	11,0	14,8	114,5
		Xall B	18,1	28,9	7,1	13,0	114,5

Table 17 shows, that in presence of C6 sugars, the xylose degradation rate of all tested yeast strains was higher than without. The presence of hexoses is therefore beneficial for xylose degradation.

Highest xylose degradation rate in presence of C6 sugars was calculated for the yeast strain C238 (Xall A 0,19 g/L/h and Xall B 0,32 g/L/h). A xylose degradation rate in the middle range was calculated for C240 and C245 with C6 sugars (0,14-0,16 g/L/h). The worst xylose degradation rates with and without C6 sugars were calculated for HA1129 (with C6 sugars 0,04 g/L/h in Xall-medium A).

Table 17: Selected yeast strains in the culture bottles with Xall-medium A and B. Overview of the calculated xylose degradation rates with and without C6 sugars.

Yeast strain	Run duration [h]	Medium	Δ Xylose with C6 sugars [g/L/h]	Δ Xylose without C6 sugars [g/L/h]	Δ Xylose without C6+ low degradation [g/L/h]
A027	497	Xall A	0,09	0,05	0,05
		Xall B	0,10	0,05	0,04
C238	476,5	Xall A	0,19	0,10	0,03
		Xall B	0,32	0,13	0,04
C240	353	Xall A	0,16	0,09	0,04
		Xall B	0,15	0,07	0,04
C245	353	Xall A	0,15	0,03	-
		Xall B	0,14	0,04	-
C255	497	Xall A	0,06	0,07	0,02
		Xall B	0,07	0,07	0,03
HA1129	503,25	Xall A	0,04	0,02	-
		Xall B	0,03	0,01	-

The highest **arabinose degradation** (see Table 16) was measured in the fermentation with A027, which was in Xall A and B over 80%, and with strain C255 which degraded around 70%. At 305 hours A027 degraded already more than 50% of the arabinose in presence of xylose in co-metabolism. In the end, A027 had degraded between 83% (in Xall A) and 90% (in Xall B) of the arabinose. The arabinose degradation of C238 in Xall A at the end of fermentation was about 39% and in Xall B approximately 60%. The experiments with the strains C240 and C245 were stopped at 353 hours and a lower amount (<35% arabinose) of arabinose was consumed.

HA1129 had a poor xylose degradation and a poor arabinose degradation, which implies that this yeast strain is not a good consumer of C5 sugars.

According to the calculated arabinose degradation rates in Table 18, the presence of xylose has no noticeable effect on the arabinose degradation. Only the data of A027 showed, that arabinose was degraded faster in the presence of C6 sugars and xylose. It must be said, that the experiments were often stopped straight after xylose was fully degraded. For this reason, no or only less data was collected for the arabinose degradation without xylose co-metabolism. The available data of C240, C245 and C255 show that the arabinose degradation rate might be faster when no other sugars are present.

Table 18: Selected yeast strains in the culture bottles with Xall-medium A and B. Overview of the calculated arabinose degradation rates with C6 sugars and xylose, with xylose and without any other sugar.

Yeast strain	Run duration [h]	Medium	Δ Arabinose with C6+Xylose [g/L/h]	Δ Arabinose with Xylose [g/L/h]	Δ Arabinose without Xylose [g/L/h]
A027	497	Xall A	0,014	0,004	-
		Xall B	0,011	0,004	-
C238	476,5	Xall A	0,003	0,003	0,001
		Xall B	0,005	0,004	0,004
C240	353	Xall A	0,001	0,001	0,005
		Xall B	0,000	0,001	-
C245	353	Xall A	0,001	0,001	0,007
		Xall B	0,003	0,001	0,001
C255	497	Xall A	0,003	0,006	-
		Xall B	0,003	0,005	0,014
HA1129	503,25	Xall A	0,001	0,001	-
		Xall B	0,001	0,001	-

The measured **optical density (OD_{600}), the cell dry mass (CDM) and the calculated biomass yield** (see chapter 4.4.9.4) is summarized in Table 19. For C240, C245 and HA1129 the optical density in the end was lower than 10 OD. C255 and A027 had in the end an OD_{600} around 20. The highest optical density in the end had the strain C238 with 36 OD.

While the CDM per OD of A027, C238, C240, C245 and C255 in Xall A and B was between 0,24-0,36 g/L/OD, the CDM of HA1129 was higher at 0,44-0,52 g/L/OD. The biomass yields of C240 and C245 were around 0,05 g CDM/g sugar consumed and therefore the lowest ones. C238 had the highest biomass yield with 0,19 g CDM/g sugar consumed (see Table 20). A027, C255 and HA1129 were in the middle range with 0,11-0,14 g CDM/g sugar.

A possible reason for the low OD and the middle biomass yield of HA1129 might be the poor conversion of xylose. Most of the converted sugars were C6 sugars. Additionally, from 1 g consumed sugar between 0,11-0,19 g cell dry mass is formed by C238, A027, C255 and HA1129, this high biomass formation was not intended in the experiments. C240 and C245 nearly completely degraded the xylose, but used the sugar for the formation of products.

Peptone and yeast extract showed no influence on the OD_{600} or the biomass yield.

Table 19: Selected yeast strains in the culture bottles with Xall-medium A and B. Overview of the OD_{600}, CDM [g/L/OD], CDM at the end of the cultivation and the biomass yield. Low biomass yield is marked green and higher one is marked red.

Yeast strain	Run duration [h]	Medium	OD_{600} 305h	OD_{600} End	CDM [g/L/OD]	CDM End [g/L]	Biomass yield [g CDM/g sugar]
A027	497	Xall A	10,9	20,5	0,27	5,5	**0,13**
		Xall B	11,4	18,5	0,25	4,8	**0,11**
C238	476,5	Xall A	23,9	36,0	0,21	7,7	**0,19**
		Xall B	25,2	36,4	0,22	8,0	**0,19**
C240	353	Xall A	5,3	7,3	0,27	2,0	**0,05**
		Xall B	7,4	7,0	0,36	2,3	**0,07**
C245	353	Xall A	4,4	5,6	0,24	1,4	**0,04**
		Xall B	4,2	5,8	0,24	1,4	**0,04**
C255	497	Xall A	11,7	21,0	0,28	5,9	**0,14**
		Xall B	12,7	20,3	0,30	6,1	**0,14**
HA1129	503,25	Xall A	3,8	5,3	0,52	2,8	**0,13**
		Xall B	3,4	5,6	0,44	2,5	**0,14**

The comparison of the CDM yield at the end of the fermentations in Table 20, calculated from the total CDM difference and the difference of all consumed sugars, is lower than the CDM yield only with the difference of the C5 sugars in the time span when all C6 sugars were consumed and the end of fermentation. As a result, the biomass yield with C5 sugars was for all yeast strains, except of C240, higher than the formed biomass from the C6 sugars.

Table 20: Selected yeast strains in the culture bottles with Xall-medium A and B (with peptone and yeast extract). Overview of the calculated CDM yields: at the END with C6+C5 sugars and between 0 g/L C6 and the END only from C5 sugars.

Yeast strain	Run duration [h]	Medium	CDM yield [g CDM /g C6+C5] END	CDM yield [g CDM/g C5] between 0g/L C6 and END
A027	497	Xall A	0,13	0,17
		Xall B	0,11	0,14
C238	476,5	Xall A	0,19	0,29
		Xall B	0,19	0,29
C240	353	Xall A	0,05	0,05
		Xall B	0,07	0,05
C245	353	Xall A	0,04	0,23
		Xall B	0,04	0,10
C255	497	Xall A	0,14	0,24
		Xall B	0,14	0,27
HA1129	503,25	Xall A	0,13	0,17
		Xall B	0,14	0,15

Table 21: Selected yeast strains in the culture bottles with Xall-medium A and B (with peptone and yeast extract). Overview of the decrease of the pH at 305 h and the end of the fermentation.

Yeast strain	Run duration [h]	Medium	Δ pH at 305h	Δ pH at End
A027	497	Xall A	-1,71	-3,11
		Xall B	-1,34	-1,92
C238	476,5	Xall A	-3,16	-3,13
		Xall B	-2,72	-2,96
C240	353	Xall A	-0,64	-0,78
		Xall B	-0,57	-0,65
C245	353	Xall A	-0,52	-0,52
		Xall B	-0,33	-0,36
C255	497	Xall A	-0,96	-3,07
		Xall B	-0,99	-2,15
HA1129	503,25	Xall A	-0,73	-1,04
		Xall B	-0,36	-0,39

Table 21 shows the **pH decrease** (from the initial pH of 6) of the strains at 305 h and at the end of cultivation. The biggest pH decrease was registered for A027, C238 and C255. C240, C245 and HA1129 lowered the pH only by a little (0,4 to 1 pH units). There was no correlation found between the drop of the pH-value and the maximum produced acetic acid. Other organic acids were not detected. It was noticed, that the decrease of the pH for Xall-medium B was for all tested yeast strains less than the pH-difference of Xall A, independent of the CDM. Maybe the buffer capacity of Xall-medium B was higher than in Xall A, because of peptone and yeast extract (Thomas et al., 2002).

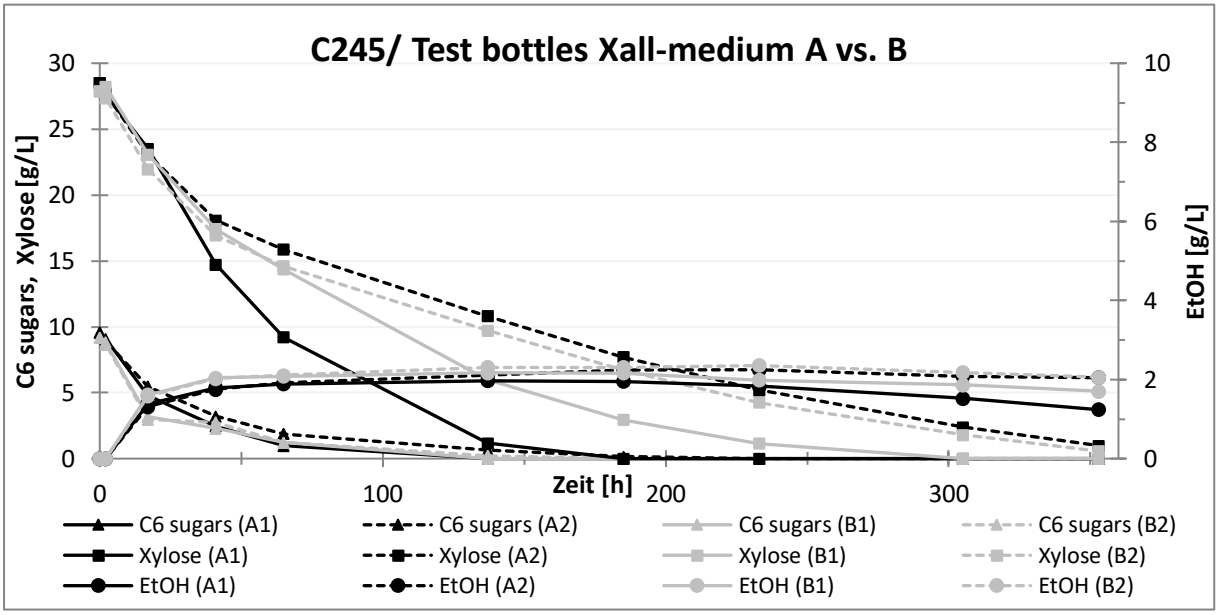

Figure 5: C245 in Xall-medium A and B. Xylose, C6 sugar and ethanol concentration is plotted, to show that ethanol from this strain is only produced from C6 sugars.

A summary of the **maximum ethanol and xylitol production** and the particular yield of all tested strains is shown in Table 22 on page 45. The yeast strains C245 and C240 showed a constant ethanol concentration, as soon as the C6 sugars were fully degraded. This means that their ethanol was merely

produced during the degradation of C6 sugars. For example, in Figure 5 the yeast strain C245 with Xall-medium A versus B with the concentrations of xylose, ethanol and the hexoses is shown. It shows, that the ethanol concentration is not further rising, after the C6 sugars were degraded. The data indicate that the produced ethanol was instead very slowly degraded by the strains.

In the test runs with yeast strains A027, C255 and C238 the concentration of ethanol increased even after all C6 sugars had been degraded. This indicates that theses strains can convert C5 sugars into ethanol. As an example for those three yeast strains, the xylose, hexose and ethanol concentration of A027 is plotted in Figure 6. The figure shows a continuously rise of the ethanol concentration, even though the C6 sugars had already been degraded.

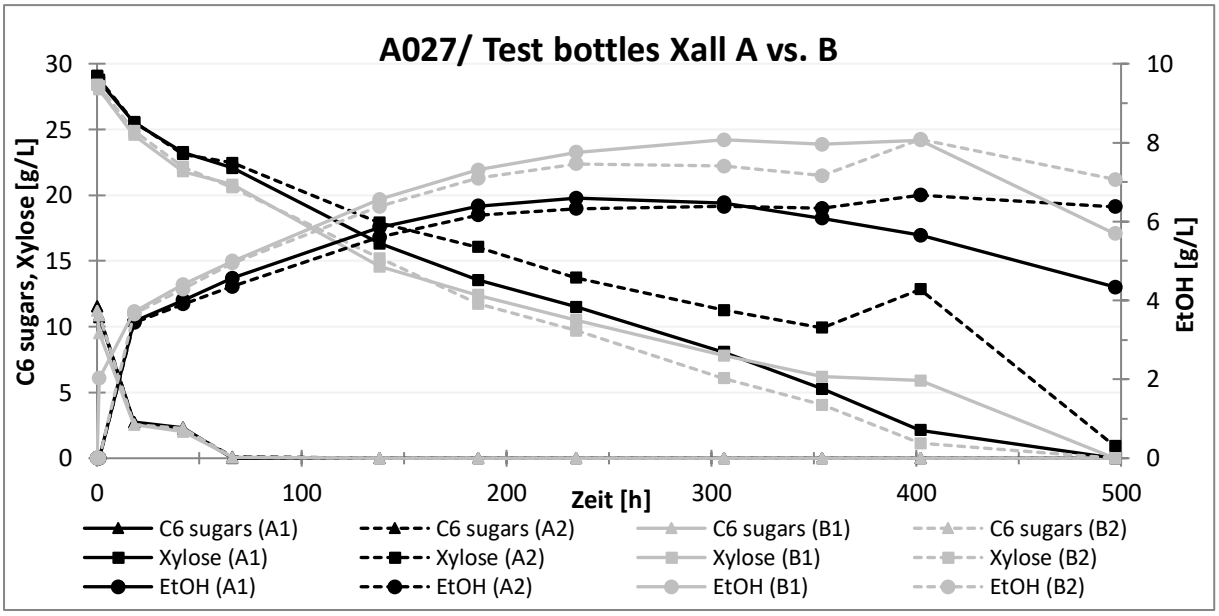

Figure 6: A027 in Xall-medium A and B. Xylose, C6 sugar and ethanol concentration is plotted, to show that ethanol from this strain is produced from C6 sugars and xylose.

The highest ethanol concentration of all test runs (8 g/L) was produced by **A027** in Xall-medium B with the maximum ethanol yield of 0,22 g ethanol per g sugar consumed (C6 plus C5 sugars). In Xall-medium A the strain A027 produced 6,6 g/L ethanol with nearly the same yield (0,21 g ethanol/g sugar). A027 also produced xylitol. The highest concentration reached was 4,96 g/L in Xall A and 5,84 g/L in Xall B. The xylitol yield (0,23-0,24 g xylitol/g xylose) was higher than the ethanol yield.

The ethanol yield (in Table 22 and Table 23 the ethanol yield at maximum ethanol concentration is calculated from all sugars, which were consumed on the point of maximum ethanol concentration (see chapter 4.4.9.2). While the xylitol yield is calculated with Formula 10 in chapter 4.4.9.3. The given ethanol yield, which was calculated from all consumed sugars by A027, is low, but the ethanol yield calculated from xylose of A027 must be much lower. Since ethanol can be produced from C6 (glucose, galactose, mannose) and C5 sugars (xylose, arabinose) a metabolic flux analysis for example with C14 marked sugars must be done to say how much ethanol was produced from which sugar (Jeffries, 2006).

Table 22: Selected yeast strains in the culture bottles with Xall-medium A and B. Maximum produced concentrations of the desired products ethanol and xylitol and the respective yield. Low max. ethanol/xylitol concentration is marked yellow and higher one is marked green.

Yeast strain	Run duration [h]	Medium	max. Ethanol [g/L]	max. Ethanol yield [g EtOH /g sugar]	max. Xylitol [g/L]	max. Xylitol yield [g Xylitol /g Xylose]
A027	497	Xall A	**6,63**	0,21	**4,96**	0,23
		Xall B	**8,08**	0,22	**5,84**	0,24
C238	476,5	Xall A	**4,11**	0,10	**10,96**	0,40
		Xall B	**5,56**	0,14	**10,49**	0,37
C240	353	Xall A	**2,85**	0,14	**19,95**	0,72
		Xall B	**3,22**	0,21	**16,18**	0,65
C245	353	Xall A	**2,11**	0,06	**22,28**	0,81
		Xall B	**2,26**	0,07	**22,54**	0,83
C255	497	Xall A	**5,79**	0,15	**13,66**	0,51
		Xall B	**4,96**	0,12	**13,25**	0,55
HA1129	503,25	Xall A	**3,54**	(0,22)	**1,61**	0,27
		Xall B	**3,72**	(0,28)	**1,49**	0,41

Table 23 shows the ethanol yield calculated from all sugars at the maximum ethanol concentration compared with the ethanol yield at the time point of fully degraded C6 sugars, calculated with C6+C5 sugars and C6 sugars. Also the ethanol yield of C5 sugars between fully degraded C6 sugars and the maximum ethanol concentration is shown in the table.

Table 23: Selected yeast strains in the culture bottles with Xall-medium A and B. Ethanol yield calculated with total sugar consumption (at the time point of maximum ethanol concentration), with C6+C5 and C6 consumption (both at 0 g/L C6 sugars) and the ethanol yield of C5 sugars between 0 g/L and the maximum ethanol concentration.

Yeast strain	Run duration [h]	Medium	Ethanol yield [g EtOH /g sugar] MAX	Ethanol yield [g EtOH /g C6+C5] at C6 0 g/L	Ethanol yield [g EtOH /g C6] at C6 0 g/L	Ethanol yield [g EtOH /g C5] between 0g/L C6 and MAX
A027	497	Xall A	0,21	0,23	0,44	0,15
		Xall B	0,22	0,25	0,58	0,16
C238	476,5	Xall A	0,10	0,19	0,36	0,03
		Xall B	0,14	0,22	0,39	0,04
C240	353	Xall A	0,14	0,17	0,29	0,03
		Xall B	0,21	0,21	0,35	-
C245	353	Xall A	0,06	0,06	0,22	-
		Xall B	0,07	0,07	0,24	0,01
C255	497	Xall A	0,15	0,16	0,37	0,13
		Xall B	0,12	0,14	0,36	0,07
HA1129	503,25	Xall A	0,22	0,25	0,36	0,01
		Xall B	0,28	0,28	0,40	-

According to the stoichiometry the maximum theoretical yield of ethanol from glucose, galactose and mannose is 0,51 gram ethanol per gram C6 sugar (Madhavan et al. (2012) and calculation see 4.4.9.2). By comparing the theoretical ethanol yield with the ethanol yield calculated with the C6 sugars (see Table 23), the yield of the yeast strains, except of A027 with Xall-medium B, was lower than

0,51 g/g. It can be excluded, that these yeast strains formed ethanol from xylose in the presence of hexose sugars. It seems like the yeasts preferred to use the C6 sugars for the formation of ethanol. This could be also the reason, why the CDM yield of the C6 sugars was lower, than for the C5 sugars (see Table 20, column on the right). After the C6 sugars were fully degraded, the only yeast strains which produced ethanol from C5 sugars with a high yield were A027 and C255 in Xall-medium A.

C238 and C255 produced between 4,11-5,79 g/L ethanol, while C238 produced 50% and C255 60% more xylitol than A027 (5-5,8 g/L xylitol). Except of C255, the maximum ethanol concentration was between 5% (for HA1129) and 35% (for C238) higher with peptone and yeast extract.

Seen from the data, HA1129 has the highest maximum ethanol yield with 0,28 g ethanol/g sugar. A closer look shows, that the high ethanol yield is a consequence of the poor xylose degradation. The data shows that the produced ethanol is almost completely produced during the degradation of C6 sugars.

C240 and C245 produced less than 3,7 g/L ethanol in both media. This shows that these strains are less suited for ethanol production compared to the strains above. C240 had an ethanol yield of 0,14-0,21 g ethanol/g sugar and C245 0,06-0,07 g ethanol/g sugar. Therefore, C240 reached a maximum xylitol concentration of 19,95 g/L in the Xall-medium A. Strain C245 reached the highest xylitol concentrations of 22,54 g/L in Xall B and 22,28 g/L in Xall A.

In Table 24 the **maximum concentrations of the side products** are listed. With exception of the strains A027 (0,5-0,7 g/L acetic acid) and HA1129 (0,3 g/L acetic acid) the strains produced less than 0,2 g/L acetic acid.

Table 24: Selected yeast strains in the culture bottles with Xall-medium A and B. Maximum produced concentrations of ethanol, xylitol, acetic acid, glycerol and arabitol/mannitol.

Yeast strain	Run duration [h]	Medium	max. Ethanol [g/L]	max. Xylitol [g/L]	max. Acetic acid [g/L]	max. Glycerol [g/L]	max. Arabitol & Mannitol [g/L]
A027	497	Xall A	6,63	4,96	0,50	1,70	1,87
		Xall B	8,08	5,84	0,69	1,51	2,04
C238	476,5	Xall A	4,11	10,96	0,13	0,68	1,30
		Xall B	5,56	10,49	0,20	0,49	1,83
C240	353	Xall A	2,85	19,95	0,00	2,09	0,73
		Xall B	3,22	16,18	0,00	1,21	0,56
C245	353	Xall A	2,11	22,28	0,00	3,45	1,31
		Xall B	2,26	22,54	0,05	2,51	1,02
C255	497	Xall A	5,79	13,66	0,00	1,35	1,88
		Xall B	4,96	13,25	0,07	1,02	1,67
HA1129	503,25	Xall A	3,54	1,61	0,31	0,28	0,53
		Xall B	3,72	1,49	0,32	0,24	0,47

The produced glycerol varies between 0,24 g/L to 3,45 g/L. The lowest maximum glycerol concentration was measured for HA1129 in Xall B. The highest maximum glycerol concentration was measured for the strain C245, with 2,5 g/L in Xall-medium B and 3,5 g/L in Xall-medium A. The other yeast strains had a maximum glycerol concentration of less than 2 g/L.

The maximum concentration of arabitol together with mannitol (same retention time in HPLC analysis, see chapter 4.4.5) varies between 0,5 g/L and 2,0 g/L. A maximum arabitol & mannitol concentration higher then 1,5 g/L was measured for A027, C238 Xall B and C255.

To summarize, **A027 (with Xall-medium B) is the best strain for ethanol production** of the tested yeast strains. The ethanol of this yeast strain was produced from C6 sugars and from the C5 sugar xylose. It produced the highest ethanol concentration and ethanol yield and less xylitol as side products. The biomass yield of A027 was in the middle range (0,11 g CDM/ g sugar) and the xylose degradation was rather slow (497 hours) but complete.

Amongst the tested yeast strains there was **no strain which produced ethanol from the pentose sugars efficiently** and with high yield. Therefore, four of the yeast strains (C238, C240, C245 and C255) produced xylitol with concentrations higher than 10 g/L and a high yield between 0,4-0,8 g xylitol/ g xylose.

The **preferred yeast strain for xylitol production is C245** with Xall-medium B. It produced the highest xylitol concentration (22,5 g/L) and had the highest xylitol yield of all tested yeast strains. With 0,83 g xylitol per g xylose, the yield is not far from the maximum theoretical yield (calculation see chapter 4.4.9.3) of 0,987 g xylitol per g xylose. 84% of xylose is converted to xylitol, while a low concentration of ethanol was formed from C6 sugars. The biomass yield was low (0,04 g CDM/ g sugar) and xylose was almost completely degraded in a short time (353 hours). A disadvantage of strain C245 is that it produces glycerol (2,5 g/L) as a side product.

The **third of the most interesting yeast strains is C238** in Xall-medium B, which was the fastest xylose degrading strain (100% within 305 h). C238 produced a maximum ethanol concentration of 5,6 g/L with a relatively low yield (0,14 g ethanol per g sugar). Ethanol was produced from C6 sugars, but also from xylose. The maximum xylitol concentration was 10,5 g/L, produced with a low xylitol yield (0,37 g xylitol per g xylose). Main disadvantages of C238 is the high biomass yield (0,19 g CDM/g sugar) and the strong pH decrease during the fermentation (decrease from pH 6 to pH 3 within 477 hours).

Strain HA1129 showed poor xylose-degradation properties in the synthetic Xall-medium, furthermore the maximum ethanol concentration was below 3 g/L. C240 had a lower maximum xylitol-concentration than C245. Therefore, the strains A027, C245 and C238 were selected for advanced testing in the fermenter with Xall-medium.

The **addition of peptone and yeast extract** to the Xall-medium showed no effect on the speed of xylose and arabinose degradation and did not influence the OD_{600} or the formation of biomass. Instead peptone and yeast extract seem to have a positive influence on the decrease of the pH-value (see Table 21) and improves the formation of ethanol (see Table 22). In the presence of peptone and yeast extract, the maximum xylitol concentration was significant higher for the yeast strain A027 and lower for C240 and HA1129.

5.1.2 Culture bottles with hydrolysate-medium A & B

In the second part of the tests in 200 mL culture bottles, only four of the yeast strains were tested with the hydrolysate-medium A and hydrolysate-medium B (peptone and yeast extract). For the media preparation and composition, have a look at chapter 4.3.3 and 4.3.4. The strains A027, C238 and C255 showed the highest ethanol concentrations and a high xylose degradation rate in the synthetic Xall-medium. In the following tests the strains were examined on ethanol-production in hydrolysate-medium. C245 had the highest concentration of xylitol in the synthetic medium and was used for xylitol production in the tests with hydrolysate-medium.

In the following tables the mean value of the duplicate testing of the strains in hydrolysate-medium A are shown together with the results of hydrolysate-medium B. The experiments were carried out with same test duration of around 475 hours. The exact duration of the experiments is also shown in the tables. For better comparability, the results at 305 hours are listed.

Table 25: Selected yeast strains in the culture bottles with hydrolysate-medium A and B. Overview of the percentage of consumed sugar at 305 hours and at the end of the experiment.

Yeast strain	Run duration [h]	Medium	Δ Xylose 305h [%]	Δ Xylose End [%]	Δ Arabinose 305h [%]	Δ Arabinose End [%]	Δ 100% Glucose at ___ hours
A027	473,5	Hydrolysate A	11,5	17,6	10,4	0,3	17,0
		Hydrolysate B	13,1	20,0	12,9	5,6	17,0
C238	473,5	Hydrolysate A	54,8	83,8	29,1	28,4	17,0
		Hydrolysate B	42,3	66,2	26,6	22,2	17,0
C245	473,5	Hydrolysate A	68,2	78,1	14,1	13,7	113,0
		Hydrolysate B	63,4	79,8	20,7	23,9	113,0
C255	475	Hydrolysate A	89,0	100,0	33,3	67,5	41,8
		Hydrolysate B	68,4	100,0	17,4	45,3	41,8

The **sugar consumption** of the yeast strains in hydrolysate-medium is listed in Table 25. The only hexose in the hydrolysate was glucose, which was fully degraded by A027, C238 within 20 hours and by C255 within 42 hours. The slowest glucose degrading strain was C245, which needed 113 hours.

The best **xylose degradation** was determined for the strain C255, which degraded 100% of xylose in both media. It also had the highest arabinose degradation of the tested strains, with 68% (1,1 g/L arabinose) with hydrolysate-medium A and 45 % (1,7 g/L) for hydrolysate-medium B.

C238 and C245 had a middle xylose degradation rate. C238 degraded the xylose faster in hydrolysate-medium A (84%, 24 g/L xylose degraded), then in hydrolysate-medium B (66%, 19 g/L xylose degraded). The arabinose degradation was between 22-28% and therefore low. C245 had in both media a xylose degradation of around 80% (around 24 g/L xylose). The total arabinose degradation was better in hydrolysate-medium B (24%) than in hydrolysate-medium A (14%). The yeast strain with the worst xylose degradation was A027. Only 20% of xylose were degraded in 474 hours, which is about 6 g/L. The arabinose concentration of A027 was nearly constant in the whole time.

Table 26: Selected yeast strains in the culture bottles with hydrolysate-medium A and B. Overview of the calculated xylose degradation rates in co-metabolism with and without C6 sugars.

Yeast strain	Run duration [h]	Medium	Δ Xylose with C6 sugars [g/L/h]	Δ Xylose without C6 sugars [g/L/h]	Δ Xylose without C6+ low degradation [g/L/h]
A027	473,5	Hydrolysate A	0,01	0,01	-
		Hydrolysate B	0,03	0,01	-
C238	473,5	Hydrolysate A	0,08	0,04	0,04
		Hydrolysate B	0,07	0,04	0,06
C245	473,5	Hydrolysate A	0,08	0,05	0,02
		Hydrolysate B	0,08	0,04	0,03
C255	475	Hydrolysate A	0,07	0,07	-
		Hydrolysate B	0,08	0,06	-

With exception of C238, peptone and yeast extract did not show an effect on substrate degradation.

Except of A027, xylose degradation rate (see Table 26) in co-metabolisms with C6 sugars of the yeast strains was between 0,7-0,8 g/L/h. While the xylose degradation was slower, when C6 sugars were fully degraded. The presence of hexoses is therefore beneficial for xylose degradation with this yeast strains. The calculated xylose degradation rate for A027 was the slowest (0,01-0,03 g/L/h) and C6 sugars had no effect on the rate.

The **arabinose degradation** rate (see Table 27) in the co-metabolism with C6 sugars (in case of hydrolysate-medium only glucose) and xylose was for all yeast strains higher than only with xylose. The experiments of A027, C238 and C245 were stopped before xylose was fully degraded. The data cannot be used to state whether the arabinose degradation rate without any co-metabolism is faster than in presence of C6 sugars and xylose. Only the fermentation of C255 ran longer and the data showed, that the arabinose degradation rate without xylose had the same speed as in co-metabolism with C6 sugars and xylose.

Table 27: Selected yeast strains in the culture bottles with hydrolysate-medium A and B. Overview of the calculated arabinose degradation rates in co-metabolism with C6 sugar and xylose, without C6 sugars and with xylose and without both.

Yeast strain	Run duration [h]	Medium	Δ Arabinose with C6+Xylose [g/L/h]	Δ Arabinose with Xylose [g/L/h]	Δ Arabinose without Xylose [g/L/h]
A027	473,5	Hydrolysate A	0,025	0,000	-
		Hydrolysate B	0,027	0,000	-
C238	473,5	Hydrolysate A	0,011	0,002	-
		Hydrolysate B	0,016	0,001	-
C245	473,5	Hydrolysate A	0,004	0,000	-
		Hydrolysate B	0,007	0,001	-
C255	475	Hydrolysate A	0,010	0,003	0,008
		Hydrolysate B	0,006	0,002	0,006

Having a look on the **optical density, cell dry mass and biomass yield** in Table 28. The CDM per OD for the yeast strains C238, C245 and C255 was very similar and between 0,29-0,35 g/L/OD. But A027 had a slightly higher CDM with 0,46-0,49 g/L/OD. There was no difference between the CDM per OD between fermentations with the two media.

While C245 had the highest biomass yield (0,28 g CDM/g sugar and max. OD 32) in hydrolysate-medium B, it also had the lowest biomass yield (0,12 g CDM/g sugar and max. OD 13) in hydrolysate-medium A. Peptone and yeast extract seem to have a strong effect on the biomass formation of C245 in hydrolysate-medium. Beside of C245, no effect of peptone and yeast extract on the formation of yeast biomass can be recognize in hydrolysate-medium.

Table 28: Selected yeast strains in the culture bottles with hydrolysate-medium A and B. Overview of the OD_{600}, CDM [g/L/OD], CDM at the end of the cultivation and the biomass yield. Low biomass yield is marked green and higher one is marked red.

Yeast strain	Run duration [h]	Medium	OD_{600} 305h	OD_{600} End	CDM [g/L/OD]	CDM End [g/L]	Biomass yield [g CDM/g sugar]
A027	473,5	Hydrolysate A	5,6	6,3	0,46	2,9	**0,16**
		Hydrolysate B	5,2	6,0	0,49	3,0	**0,15**
C238	473,5	Hydrolysate A	23,3	28,8	0,33	9,5	**0,25**
		Hydrolysate B	15,4	25,7	0,34	8,7	**0,26**
C245	473,5	Hydrolysate A	6,7	13,4	0,35	4,5	**0,12**
		Hydrolysate B	26,5	31,5	0,34	10,8	**0,28**
C255	475	Hydrolysate A	13,5	28,2	0,29	8,2	**0,25**
		Hydrolysate B	15,2	29,5	0,30	8,7	**0,28**

A high optical density (26-30 OD_{600}) and biomass yield (0,25-0,28 g CDM/g sugar) was determined for C255 and C238 in both hydrolysate-media. The OD_{600} of A027 was around OD 6 and therefore the lowest of the tested yeast strains. Consequentially the biomass yield of A027 was also low, with 0,15-0,16 g CDM/g sugar. Regarding the CDM yield (see Table 29), the fermentation in hydrolysate showed the same effect as in the Xall-medium (see chapter 5.1.1, Table 20). The CDM yield of the C5 sugars was for all four yeast strains higher than the CDM yield of the C6 sugars.

Table 29: Selected yeast strains in the culture bottles with hydrolysate-medium A and B. Overview of the calculated CDM yields: at the END with C6+C5 sugars and between 0 g/L C6 and the END from C5 sugars.

Yeast strain	Run duration [h]	Medium	CDM yield [g CDM /g sugar] END	CDM yield [g CDM/g C5] between 0g/L C6 and END
A027	473,5	Hydrolysate A	0,16	0,49
		Hydrolysate B	0,15	0,39
C238	473,5	Hydrolysate A	0,25	0,35
		Hydrolysate B	0,26	0,42
C245	473,5	Hydrolysate A	0,12	0,20
		Hydrolysate B	0,28	0,53
C255	475	Hydrolysate A	0,25	0,32
		Hydrolysate B	0,28	0,36

During the fermentation, the **pH** dropped only between 0,4 and 1,1 pH units (see Table 30) in all test runs. There was no correlation found between the pH drop and the other data. The hydrolysate ingredients seem to have a positive influence on the pH-value, due to the lower decrease in the hydrolysate-media than in the Xall-medium (see chapter 5.1.1, Table 21).

Table 30: Selected yeast strains in the culture bottles with hydrolysate-medium A and B. Overview of the CDM [g/L] and the decrease of the pH at 305 h and the end of the fermentation.

Yeast strain	Run duration [h]	Medium	Δ pH 305h	Δ pH End
A027	473,5	Hydrolysate A	-0,89	-0,97
		Hydrolysate B	-0,93	-1,08
C238	473,5	Hydrolysate A	-0,72	-0,87
		Hydrolysate B	-0,58	-0,73
C245	473,5	Hydrolysate A	-0,62	-0,81
		Hydrolysate B	-0,72	-0,87
C255	475	Hydrolysate A	-0,30	-0,42
		Hydrolysate B	-0,33	-0,51

The **product concentrations** are listed in Table 31. The highest ethanol concentration of the yeast strains was 5,93 g/L, which was measured at C238 with hydrolysate-medium B. The max. ethanol concentration of C238 in hydrolysate-medium A was slightly lower with 5,59 g/L. The biggest part of the ethanol seems to be formed from the glucose in the medium, but also a small amount was formed from xylose. C238 also formed a low amount of xylitol (around 2 g/L) in both media with a xylitol yield of 0,08-0,09 g xylitol/g xylose.

Table 31: Selected yeast strains in the culture bottles with hydrolysate-medium A and B. Maximum produced concentrations of the desired products ethanol and xylitol and the respective yield. Low max. ethanol/xylitol concentration is marked yellow and higher one is marked green.

Yeast strain	Run duration [h]	Medium	max. Ethanol [g/L]	max. Ethanol yield [g EtOH /g sugar]	max. Xylitol [g/L]	max. Xylitol yield [g Xylitol /g Xylose]
A027	473,5	Hydrolysate A	**3,98**	0,27	**0,16**	0,06
		Hydrolysate B	**4,28**	0,26	**0,19**	0,04
C238	473,5	Hydrolysate A	**5,59**	0,16	**1,95**	0,08
		Hydrolysate B	**5,93**	0,21	**1,82**	0,09
C245	473,5	Hydrolysate A	**2,79**	0,13	**13,39**	0,64
		Hydrolysate B	**3,40**	0,14	**6,35**	0,31
C255	475	Hydrolysate A	**2,45**	0,08	**7,77**	0,32
		Hydrolysate B	**1,96**	0,08	**4,34**	0,21

The second highest ethanol concentration was reached by A027. While the maximum ethanol concentration for hydrolysate-medium A was 3,98 g/L, it was 4,28 g/L for the medium with peptone and yeast extract. The ethanol yield of A027 is by way of calculation the highest (0,26-0,27 g ethanol/g sugar), but read out from Figure 7 most of the ethanol was produced from glucose.

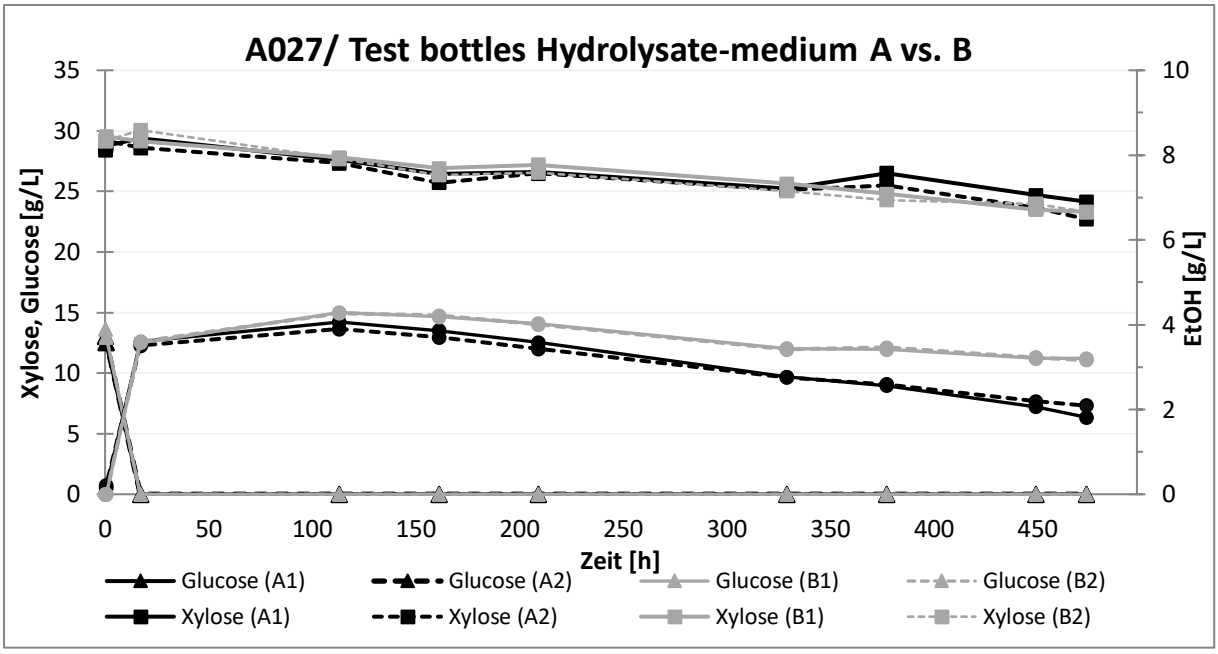

Figure 7: A027 in hydrolysate-medium A and B. Xylose, glucose and ethanol concentration is plotted, to show that the main part of the ethanol from this strain is produced from glucose.

The fact, that xylose degradation of A027 is poor, is also reflected in the low maximum xylitol concentration of 0,16-0,19 g/L and the lowest xylitol yield (0,04-0,06 g xylitol/g xylose). With a low xylose degradation and the missing conversion of xylose into ethanol in hydrolysate-medium, A027 becomes irrelevant for the following tests.

The ethanol yield of C238 in hydrolysate-medium B is 0,2 g ethanol/g sugar and therefore the highest, but it is still not high enough for an economic usage. Here the same problem (see chapter 5.1.1) occurs: Since ethanol can be produced from C6 (glucose) and C5 sugars (xylose, arabinose), further analysis (like metabolic flux analysis with C14 sugars (Jeffries, 2006)) needs to be done to know how much ethanol was built from the glucose and which was built from xylose.

Table 32: Selected yeast strains in the culture bottles with hydrolysate-medium A and B. Ethanol yield calculated with total sugar consumption (at the time point of maximum ethanol concentration), with C6+C5 consumption and C6 consumption (both at 0 g/L C6 sugars) and the ethanol yield of C5 sugars between 0 g/L and the maximum ethanol concentration.

Yeast strain	Medium	Ethanol yield [g EtOH /g sugar] MAX	Ethanol yield [g EtOH /g C6+C5] at C6 0 g/L	Ethanol yield [g EtOH /g C6] at C6 0 g/L	Ethanol yield [g EtOH /g C5] between 0g/L C6 and MAX
A027	Hydrolysate A	0,27	0,25	0,27	0,44
	Hydrolysate B	0,26	0,24	0,26	0,46
C238	Hydrolysate A	0,16	0,25	0,28	0,10
	Hydrolysate B	0,21	0,24	0,27	0,17
C245	Hydrolysate A	0,13	0,13	0,21	-
	Hydrolysate B	0,14	0,14	0,24	0,03
C255	Hydrolysate A	0,08	0,15	0,23	0,05
	Hydrolysate B	0,08	0,15	0,23	0,04

When the theoretical ethanol yield is compared to the ethanol yield calculated with the C6 sugars (see Table 32), in the presence of C6 sugars none of the yeast strains had an ethanol yield with 0,51 g/g or higher. Similar to the experiments with Xall-medium (see chapter 5.1.1) the yeasts seem to prefer the use of C6 sugars for the formation of ethanol and not for biomass (compare with Table 29). This means that the biggest part of the produced ethanol was formed from the glucose in the hydrolysate-medium at the beginning of the fermentation. The only high ethanol yield from C5 sugars after the fully assimilation of the C6 sugars was A027 (0,44-0,46 g ethanol/g C5 sugars), but due to the poor xylose degradation, the formed ethanol concentration was still too low. To sum up, none of yeast strains produced ethanol from the pentose sugars in the hydrolysate-medium efficiently.

Instead of ethanol, the yeast strains C245 and C255 produced xylitol from xylose with concentrations higher than 4 g/L and a yield between 0,2-0,6 g xylitol/ g xylose. The highest xylitol concentration of 13,39 g/L and xylitol yield of 0,64 g xylitol/g xylose was measured from C245 in hydrolysate-medium A. C245 in hydrolysate-medium A formed a low maximum ethanol concentration of 2,8 g/L. Meanwhile C245 in hydrolysate-medium B only produced a maximum xylitol concentration of 6,35 g/L with a yield of 0,31 g/g, but a higher maximum ethanol concentration of 3,40 g/L. In both media, the ethanol of C245 was only formed in the beginning of the fermentation from the contained glucose. This can also be shown by the maximum ethanol yield of C245 in hydrolysate-medium A and B, which was with 0,13 g/g and 0,14 g/g similar.

The maximum xylitol concentration of C255 was for hydrolysate-medium A higher (7,8 g/L) than for medium B (4,3 g/L). It seems peptone and yeast extract do have the same negative effect on the xylitol formation of C245 and C255. This effect leads to a approx. 50% reduction of the xylitol concentration. Instead peptone and yeast extract seem to have a positive effect on the growth of C245, because the yeast formed 50% more biomass compared to the fermentation without peptone and yeast extract (see Table 28).

Nevertheless, the xylitol yield of C255 is half of the yield of C245. For C255 the lowest maximum concentration of ethanol (2-2,5 g/L) and the lowest ethanol yield (0,08 g ethanol/g sugar) was measured. The observation shows, that the main part of the ethanol is formed from the glucose in hydrolysate-medium (like in Figure 7 for yeast strain A027).

The **side product formation** Table 33 of A027, C238 and C255 was low. The maximum acetic acid concentration for A027, C238 and C245 was the same (1,45 g/L in hydrolysate-medium A and 1,26 g/L in hydrolysate-medium B), because the acid came from the hydrolysate in the medium (see chapter 4.3.4). In the fermentation with C255 the maximum acetic acid in the hydrolysate-medium was lower with 0,4 g/L. No acetate was formed from the yeasts, they rather metabolized the acetic acid.

The maximum glycerol concentration of A027, C238 and C255 was between 0,7-1,6 g/L. While C238 had the lowest and A027 the highest glycerol concentration. The yeast strains C245 formed a higher maximum concentration of glycerol. With peptone and yeast extract the glycerol concentration was 2,14 g/L, but without it was 4,32 g/L.

The arabitol and mannitol concentration of A027, C238 and C245 was between 0,2 g/L and 0,5 g/L. C255 had a slightly higher maximum arabitol and mannitol concentration of around 1 g/L.

Table 33: Selected yeast strains in the culture bottles with hydrolysate-medium A and B. Maximum produced concentrations of ethanol, xylitol, acetic acid, glycerol and arabitol/mannitol.

Yeast strain	Run duration [h]	Medium	max. Ethanol [g/L]	max. Xylitol [g/L]	max. Acetic acid [g/L]	max. Glycerol [g/L]	max. Arabitol & Mannitol [g/L]
A027	473,5	Hydrolysate A	3,98	0,16	1,45	1,55	0,21
		Hydrolysate B	4,28	0,19	1,26	1,64	0,23
C238	473,5	Hydrolysate A	5,59	1,95	1,45	0,70	0,33
		Hydrolysate B	5,93	1,82	1,26	1,10	0,24
C245	473,5	Hydrolysate A	2,79	13,39	1,45	4,32	0,54
		Hydrolysate B	3,40	6,35	1,26	2,14	0,43
C255	475	Hydrolysate A	2,45	7,77	0,44	1,32	1,06
		Hydrolysate B	1,96	4,34	0,43	1,22	0,97

Conclusion: A027 is unproductive in hydrolysate-medium, because it has a poor xylose degradation and converts only glucose to ethanol. For this reason, A027 is not used for further tests.

C238 forms a lot of biomass and has a high biomass yield, but still had the highest ethanol concentration and formed less xylitol. The higher maximum ethanol concentration might come from the higher glucose concentration of (7-10 g/L glucose in the hydrolysate; 3 g/L glucose, 3 g/L galactose and 3 g/L mannose in the Xall-medium) the hydrolysate-medium.

C255 degrades xylose completely, but forms a lot of biomass (biomass yield 0,25-0,28 g CDM/g sugar) from it, instead of the required products. The maximum ethanol concentration was below 2,5 g/L and therefore the lowest of all tested yeast strains. The maximum xylitol concentration of C255 was half of the concentration formed by C245. For the future experiments the more efficient yeast strains A027 and C238 were chosen for ethanol production and C245 was chosen for xylitol production.

Except of C238 the **addition of peptone and yeast extract** the hydrolysate-medium in the culture bottles showed no effect on the substrate degradation. Also for all yeast strains no change in the decrease of the pH-value in the presence of peptone and yeast extract was proven.

Similar to the small-scale experiments with Xall-medium, the maximum ethanol concentration, for all yeast strains except of C255, was higher with peptone and yeast extract (between 6% for C238 and 22% for C245). Instead the presence of peptone and yeast extract seems to have a negative effect on the xylitol formation of C255. For C245 peptone and yeast extract in the medium seem to increase the optical density (high biomass yield 0,28 g CDM/g sugar) and decrease the xylitol-yield (0,31 g xylitol/g xylose) and the glycerol formation (2,14 g/L).

5.2 Test runs in the fermenter

For the scale-up experiments, the 1L-fermenters were set up and started as described in chapter 4.2.3. Only the strains with higher product-concentrations in the test bottles were further tested.

To verify the reproducibility, the fermentations were done two times consecutively. The fermentations were always started with medium, which contained 30 g/L xylose (preparation described in chapter 4.3.2 and 4.3.4). When xylose was degraded to a certain concentration, more xylose, arabinose and for the hydrolysate-experiments also glucose was added to the culture broth for a fed-batch-process with a total xylose concentration around 100 g/L in the feed-part. The fed-batch process is described in chapter 4.2.3.4.

The fermenters were sampled each working day (see chapter 4.4.2). The optical density (OD_{600}, see chapter 4.4.3), pH-value (chapter 4.4.3) and the amounts of sugars and metabolic products (by HPLC, see chapter 4.4.5) were measured. Once a week the samples were microscopically examined (chapter 4.4.8). The experiment runs were run until the added xylose was completely metabolized. In case of poor and slow xylose degradation, the experiments were stopped earlier. The cell dry mass (CDM, see chapter 4.4.7) was determined, before the sugars were added again and when the experiments were stopped.

5.2.1 Xall medium in fed-batch

First the runs in synthetic Xall-medium (see chapter 4.3.2) were carried out. The strains which showed the highest ethanol production in the culture bottles with Xall-medium were the strains A027 and C238. As third strain, C245 was tested for xylitol production in the fermenter.

In the culture bottles peptone & yeast extract had a positive effect on the fermentation of A027, C238 and C245 with Xall-medium. For this reason, the yeast strains were tested on synthetic Xall-medium B with peptone & yeast extract (see chapter 4.3.2) in the fermenter.

5.2.1.1 A027/ *Saccharomyces cerevisiae* BP10001

The two consecutive fermentation runs with A027 (Xall-medium B), gave the same results, which shows that the runs are reproducible (see Figure 8 to Figure 11).

Figure 8 shows the measured **optical density (OD_{600}) and the pH-values** over the fermentation time. Up to hour 200 the optical density increases from 1,8 to an OD of 15. Afterwards the OD was constant, while the xylose was further degraded to 10 g/L. When the sugar feed was added (run 1: 325 hours and run 2: 360 hours), the fermentation broth was diluted. The OD is decreased by the dilution and increases afterwards for roughly 1,5 OD-units. The optical density is not further increased after the sugar-feed.

Through the fermentation time (489 hours) the pH-value dropped from a pH of 6 to a pH of 4.

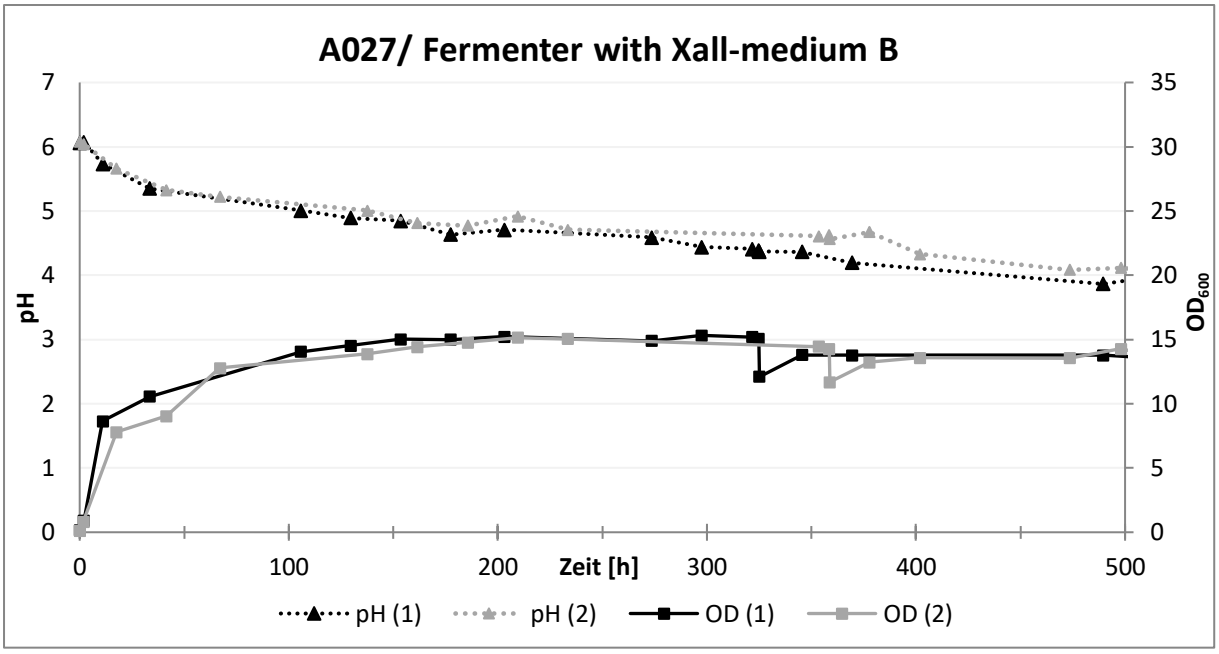

Figure 8: Measured optical density (OD) & pH-value of strain A027 with Xall-medium B

The **degradation of the substrates** contained in the Xall-medium B is showed in Figure 9. The present hexoses – glucose, galactose and mannose – were consumed within about 17 hours in both runs. Approximately 30 g/L xylose was present at the beginning of the fermentation. The degradation behavior of the first 30 g/L xylose was in both runs the same. In the first run 25 g/L of 29 g/L xylose were degraded in 325 hours with a rate of 0,08 g/L/h. In the second run 25 g/L xylose of 28 g/L were degraded in 358 hours with a rate of 0,07 g/L/h.

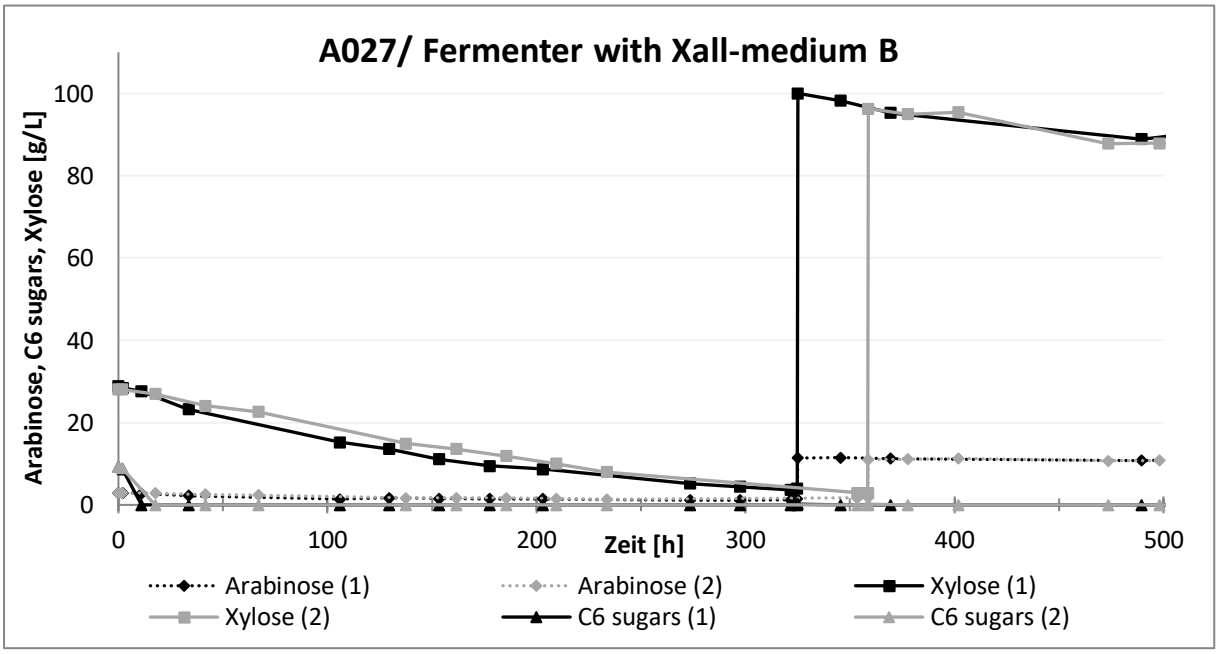

Figure 9: Substrate concentration of strain A027 with Xall-medium B

To obtain a concentration of 100 g/L xylose and 10 g/L arabinose a substrate feed was added around 325 hours (run 1) and 360 hours (run 2), when the first xylose was almost degraded. After the addition of the feed, the degradation in the first run was slower. About 13 g/L xylose of 100 g/L were degraded in 309 hours which results in a rate of 0,04 g/L/h. While in the second run 13 g/L xylose of

96 g/L were degraded in 187 hours, resulting in a rate of 0,07 g/L/h. Due to time constraints, the fermentations were stopped at 500 hours.

Over the whole fermentation time the **concentration of arabinose** hardly changed. For the batch-part of both runs 30-50% of the arabinose were degraded. In both runs during the feed-part, around 5% arabinose was degraded.

The two yeast products **ethanol and xylitol**, are plotted over fermentation time in Figure 10. The drop in xylitol and ethanol concentrations at 325 and 360 hours marks the addition of the sugar feed and the dilution of the fermentation broth.

In the first run with 34,5 g/L xylose and the hexoses, 10,9 g/L ethanol hours (regarding for dilution by the feed, undiluted max. produced ethanol 12,5 g/L) were formed in 498 hours. In the second run an ethanol concentration of 11,6 g/L (undiluted 13,4 g/L) was formed in 498 hours with the hexoses and 33,6 g/L xylose. In both runs about 4 g/L ethanol were formed while the C6 sugars in the batch were degraded. It can be assumed, that this amount of ethanol is formed from the hexoses only. However, the ethanol concentration still increased afterwards, which means that the yeast strain A027 also converted xylose to ethanol. After the addition of the sugar feed, the ethanol formation rate was lower than before, but it seems like the ethanol concentration would still rise, if the fermentation was continued.

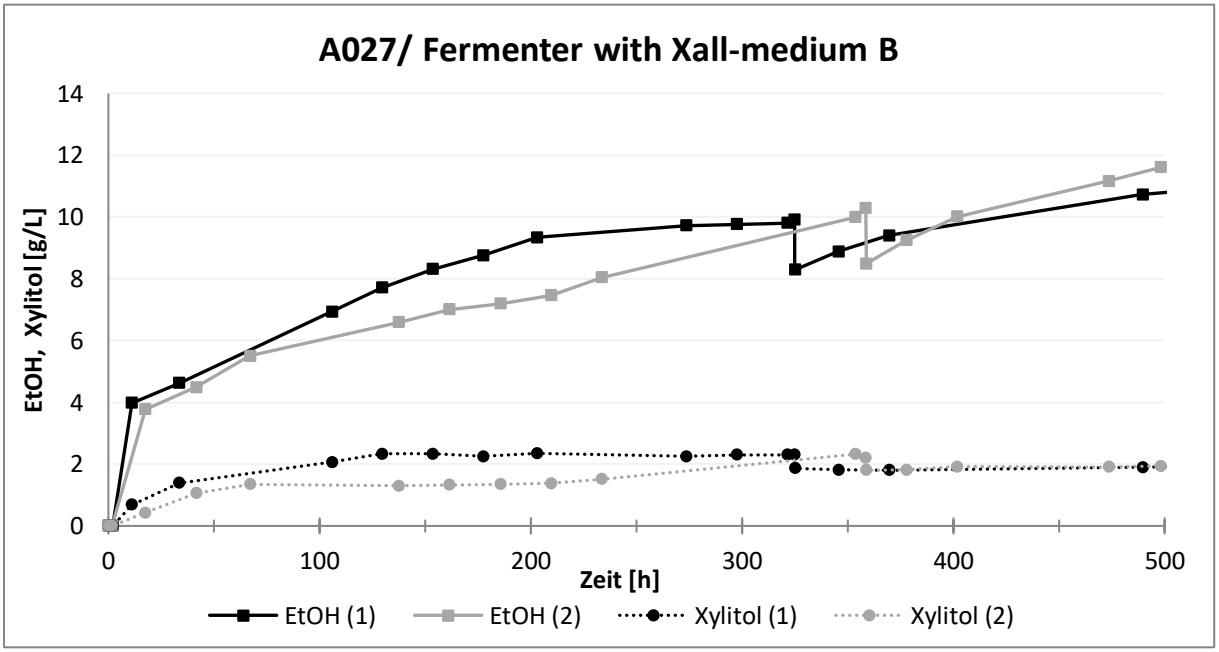

Figure 10: Intended products ethanol & xylitol of strain A027 with Xall-medium B

At the same time the xylitol concentration in the first run reached only a low concentration of 2,4 g/L at 203 hours (batch part) with 20,1 g/L consumed xylose. In the second run 2,3 g/L xylitol was formed in 354 hours (batch part) with 25,2 g/L consumed xylose. The xylitol concentration reached a maximum and was constant afterwards. Also the addition of the xylose feed (from 2,9 to first run 100 g/L and second run 96 g/L) could not increase the xylitol production.

Figure 11 shows the **concentrations of the side products** of A027 in Xall-medium B during the fermentation. In both runs the acetate concentration was less than 0,5 g/L (in 500 hours), while

glycerol reaches maximum 1 g/L. The highest side product concentration was reached by arabitol and mannitol, which had a maximum concentration of around 1,5 g/L.

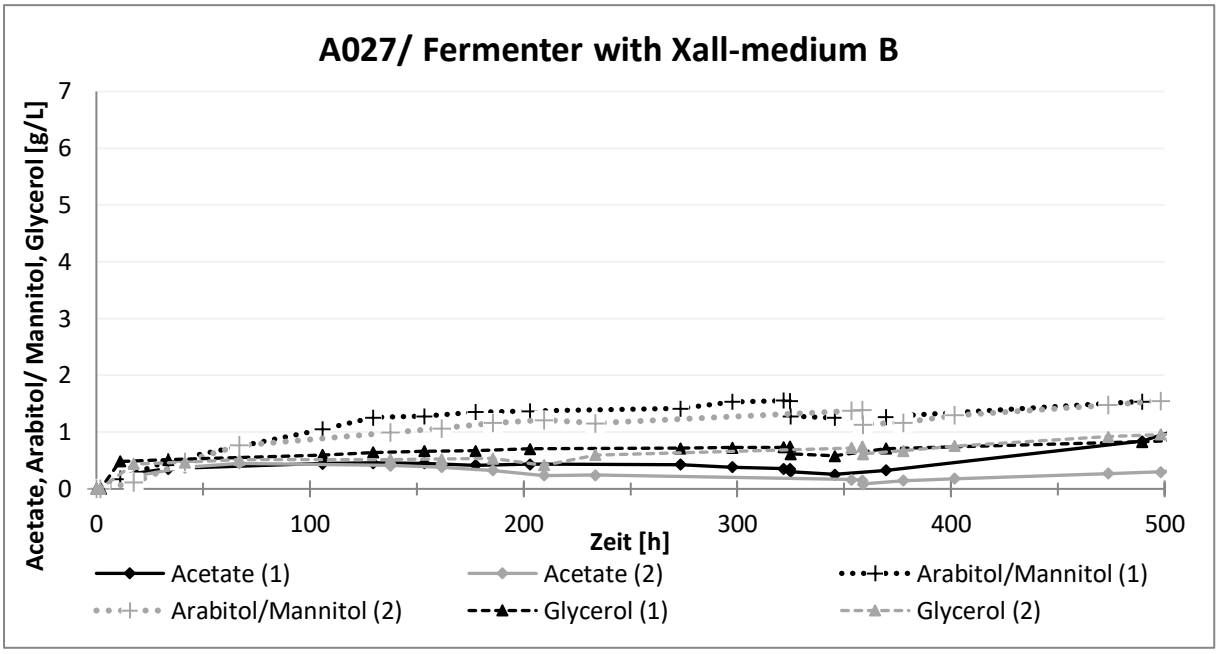

Figure 11: Side products of strain A027 with Xall-medium B

5.2.1.2 C238/ unknown strain

The curves of the two consecutive runs of the fermentation of C238 (see Figure 12 to Figure 15) differ considerably. This is most likely because of a problem with the clogged air-sparger and the broken aeration tube.

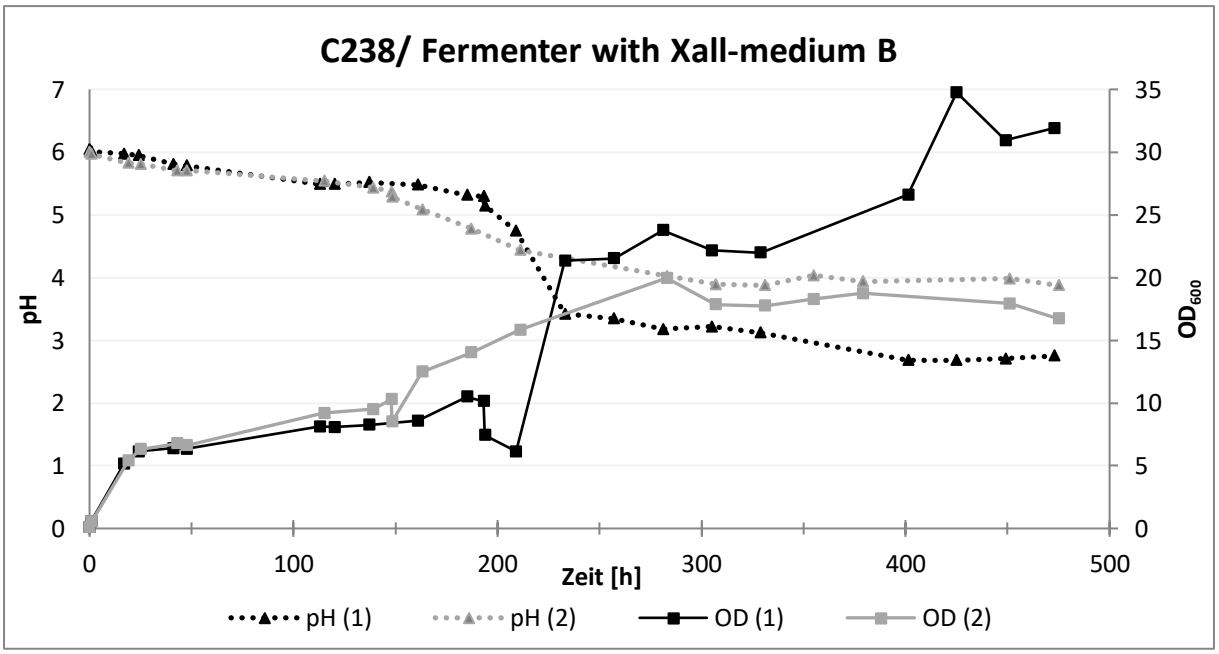

Figure 12: Measured optical density (OD) & pH-value of strain C238 with Xall-medium B

Especially the curves of **the optical density and the pH** (see Figure 12) were not similar. In the batch, the OD increased in both runs up to around 10 OD. After the addition of 100 g/L xylose and 10 g/L arabinose at 193 hours (run 1) and 148 hours (run 2), the optical density increased from 6,1 to

a maximum of 34,8 in the first run and in the second run from an initial OD of 8,5 only to 20,0. The pH dropped from the initial value of 6 to pH 2,8 in the first and to 3,9 in the second run.

Figure 13 shows the **substrate consumption** over time. The C6 sugars in the first run were degraded at around 40 hours (mannose degradation was slower than the degradation of the other C6 sugars) and in the second run at around 20 hours.

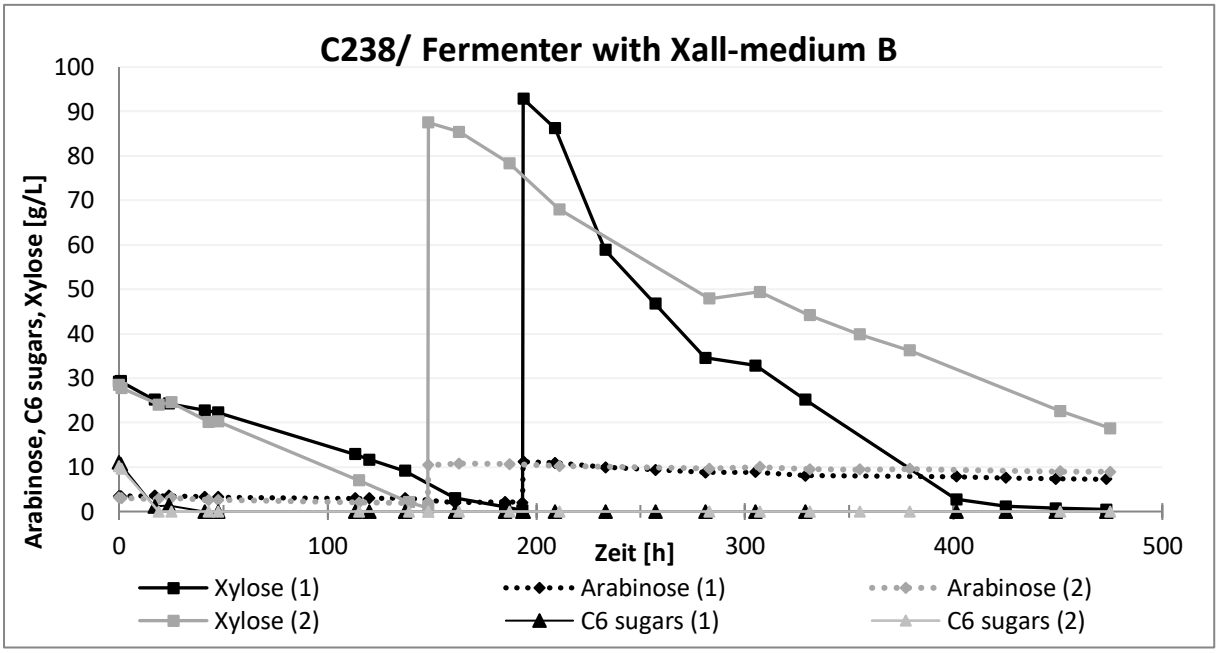

Figure 13: Substrate concentration of strain C238 with Xall-medium B

In the batch, the degradation behavior of the 30 g/L **xylose** was similar and had nearly the same slope. While in the first run 29 g/L xylose were degraded in 193 hours with a rate of 0,15 g/L/h, the 28 g/L xylose in the second run were degraded in 148 hours with a rate of 0,19 g/L/h. The degradation in the second batch run was therefore slightly faster.

For the feed xylose and arabinose were added again at 194 hours (first run) and at 148 hours (second run), when xylose was almost degraded. After the feed, 92 g/L of 92,9 g/L were degraded in 279 hours in the first run. In the second run 68,8 g/L of 87,5 g/L were degraded in 327 hours. The average xylose degradation rate of the first was therefore 0,33 g/L/h and of the second run was 0,21 g/L/h. The first run was 36% faster than the second run. A possible reason is the higher amount of formed biomass of the yeasts in the first run. Table 34 and Table 35 show the xylose degradation rate in [g xylose/g CDM/h], [g xylose/h] and specific in [g/g CDM] of the first and second fermentation run. The slower xylose degradation in the second run can also be indicated by the lower xylose degradation rates in [g/L/h] in Table 34 and Table 35.

The xylose degradation rates per CDM change over the fermentation time. The highest xylose degradation rates per CDM were reached in the batch-part with a CDM between 1,7-2,5 g/L and in the feed-part with a CDM around 4,5 g/L. In the batch-part of the first run the rate was around 7,81 g xylose/g CDM and 0,08 g xylose/g CDM/h. In the second run the specific xylose degradation rate was doubled with 13,35 g xylose/g CDM, while the degradation rate per hour was the same with 0,09 g xylose/g CDM/h. In the feed-part with 4,5 g/L CDM, the yeast strain C238 in the first run reached

a specific degradation rate of 4,67 g xylose/g CDM and 0,15 g xylose/g CDM. In the second run the specific degradation rate was 4,63 g xylose/g CDM and 0,07 g xylose/g CDM/h.

Table 34: Xylose degradation rate per CDM [g/ g CDM/ h], specific in [g/g CDM] and [g/h] in the first run of C238 in the fed-batch process in the fermenter with Xall-medium B.

Process	Ø CDM [g/L]	Δ Duration [h]	Δ Xylose [g/L]	Δ Xylose [g/g CDM]	Δ Xylose [g/g CDM/h]	Δ Xylose [g/L/h]
Batch	1,66	137,00	20,09	12,105	0,088	0,147
	2,53	56,25	8,91	3,516	0,063	0,158
Feed	4,34	87,25	58,26	13,424	0,154	0,668
	6,21	24,00	1,74	0,280	0,012	0,073
	6,38	96,25	30,16	4,725	0,049	0,313
	8,40	71,75	2,26	0,269	0,004	0,032
			Mean value	**5,720**	**0,062**	**0,232**

Table 35: Xylose degradation rate per CDM [g/ g CDM/ h], specific in [g/g CDM] and [g/h] in the second run of C238 in the fed-batch process in the fermenter with Xall-medium B.

Process	Ø CDM [g/L]	Δ Duration [h]	Δ Xylose [g/L]	Δ Xylose [g/g CDM]	Δ Xylose [g/g CDM/h]	Δ Xylose [g/L/h]
Batch	2,07	148,00	27,64	13,346	0,090	0,187
Feed	3,19	14,75	2,05	0,642	0,044	0,139
	4,72	120,00	37,54	7,950	0,066	0,313
	5,51	192,00	29,18	5,295	0,028	0,152
			Mean value	**6,808**	**0,057**	**0,198**

The mean specific xylose degradation per CDM in the second run is higher, while the degradation rate in [g xylose/g CDM/h] is similar in both runs. These data show that the speed of the degradation is not only dependent on the higher cell dry mass in the fermenter, but also on the fermentation time and the xylose concentration in the fermenter.

In both runs, the xylose degradation curves in the diagram show a decrease in xylose degradation at around 300 hours. This bend seems to be a consequence of the worse air-input, when the glass frit became overgrown and had to be cleaned. It also shows, that decrease in air-input might deteriorate the xylose degradation.

The concentration of **arabinose** changed only little over the fermentation time. In the first run around 38% in the batch and feed part were degraded. In the second run was the degradation of the arabinose 33% in the batch part and 15% in the feed part of the process. In Figure 13 the arabinose degradation rate seems faster when the xylose concentration was lower (discussed in detail in the comparison in chapter 5.2.1.4 in Table 38).

The rise of the **products ethanol and xylitol** over fermentation time is shown in Figure 14. The ethanol concentration in both runs increased slowly but continuously. The ethanol was therefore formed from hexoses and xylose. In the first run the maximum ethanol concentration formed from 121,4 g/L xylose and the contained hexoses was 16 g/L in 473 hours (regarding for dilution by the feed, max. produced ethanol 16,5 g/L). In the second run only 9,5 g/L ethanol (undiluted 10,1 g/L) from

96,4 g/L xylose and the hexoses was formed in 475 hours. The most ethanol from xylose was formed after the addition of xylose and arabinose in the feeding part of the process. The ethanol formation was inefficient compared to the xylitol formation. The maximum xylitol concentration in the first run was 50 g/L (undiluted 50,9 g/L) from 121,2 g/L xylose (in 449 hours) and in the second run 52,1 g/L (undiluted 54,8 g/L) (in 475 hours) from 96,4 g/L xylose. In the first run at 401 hours, the xylose concentration was so low (3 g/L), that the yeast started to metabolize the formed xylitol.

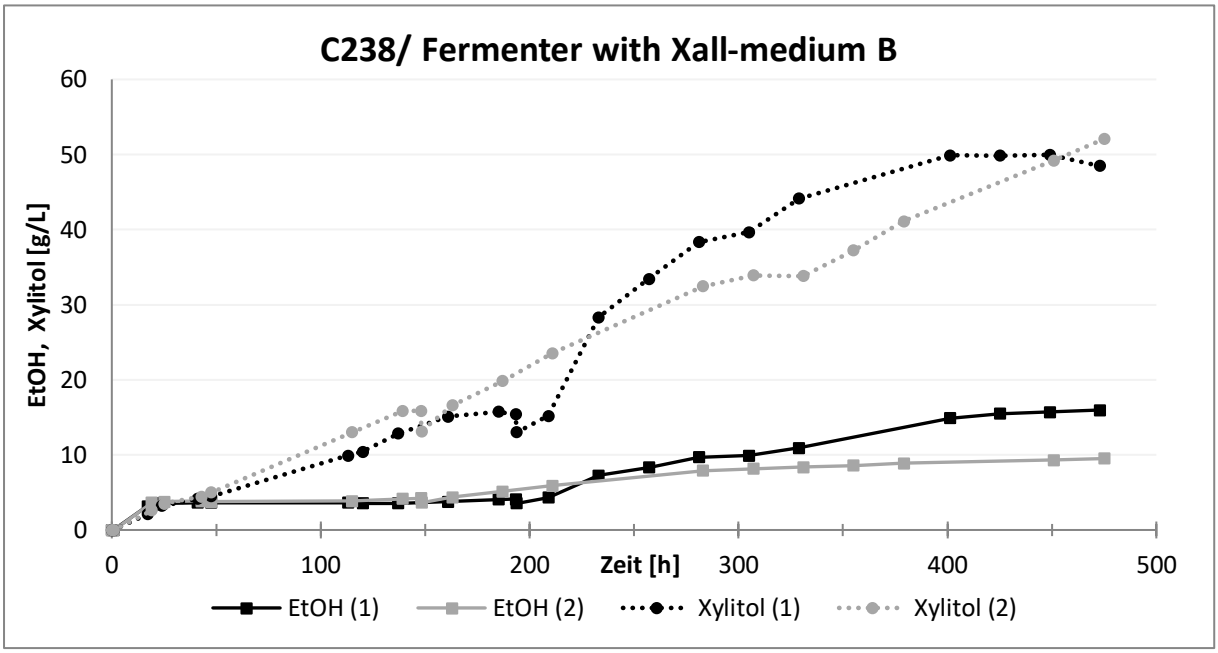

Figure 14: Intended products ethanol & xylitol of strain C238 with Xall-medium B

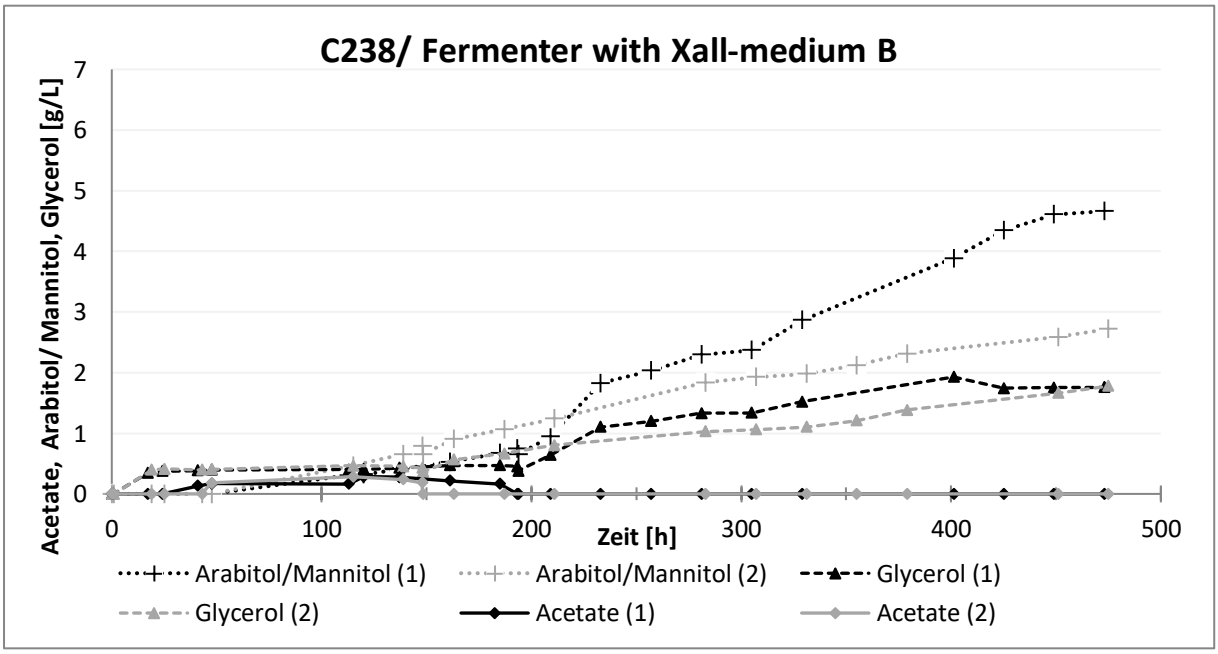

Figure 15: Side products of strain C238 with Xall-medium B

The **side product formation** (see Figure 15) for glycerol and the acetic acid was low, while the concentration of arabitol and mannitol was higher. The maximum glycerol concentration in the first

run was 1,9 g/L at 400 hours and 1,78 g/L in the second run at 475 hours. Less than 0,3 g/L acetic acid was formed before xylose was added again and afterwards no acetic acid was formed.

The maximum arabitol and mannitol concentration in the first run reached 4,7 g/L and in the second run 2,7 g/L at the end of the fermentation. The stronger degradation of arabinose due to the earlier full degradation of xylose could be a possible consequence of the twofold arabitol and mannitol concentration in the first run. It can be assumed that the measured peak at the same retention time is only arabitol.

5.2.1.3 C245/ *Candida* sp.

All curves of the two consecutive fermentation runs, in Figure 16 to Figure 19, have a good comparability, which shows the good reproducibility of the fermentation.

The increase of the **optical density** (see Figure 16) was in both runs low. In the batch part (30 g/L xylose at start), the yeast cells rose up to an OD of 4,5 and in the feed part (100 g/L xylose) the OD reached a value of 7,5 (first run) and 7,2 (second run). The sudden increase of the end OD from 5,2 to 7,2 in the second run, comes from the formed biomass at the inner fermenter wall which was rinsed off for the last measurement.

The **pH-value** (see Figure 16) decreased from a pH of 6 to a pH of 4,5 (first run) and 5 (second run).

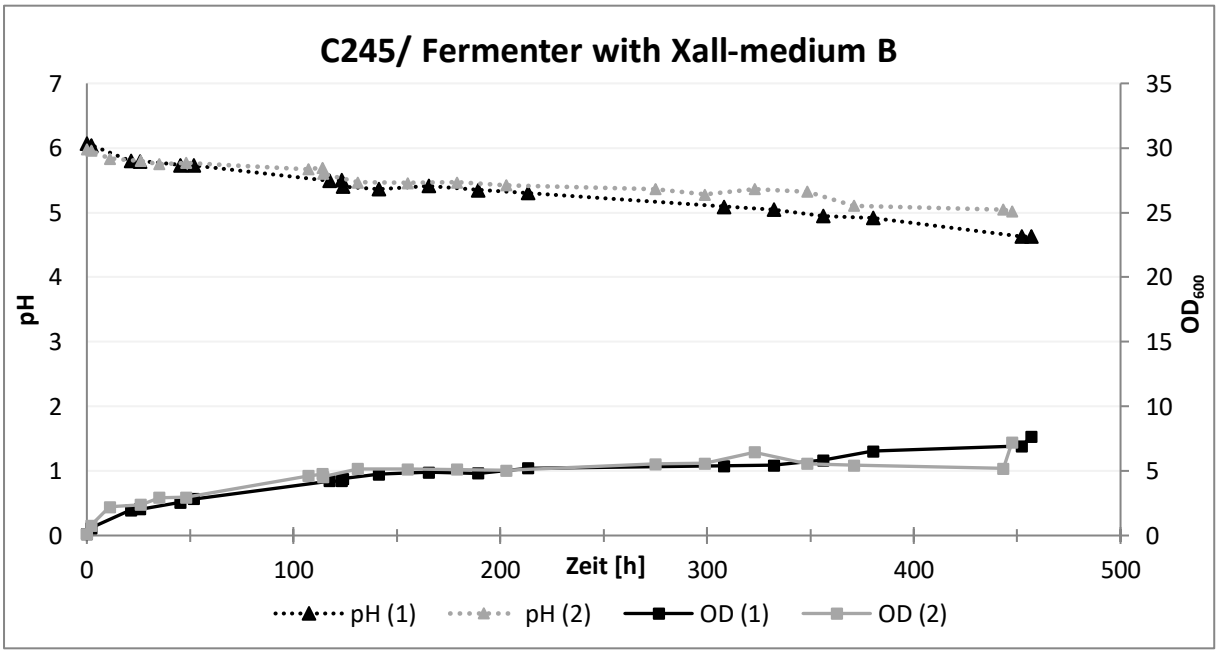

Figure 16: Measured optical density (OD) & pH-value of strain C245 with Xall-medium B

The **C6 sugars** were degraded in 140 hours (see Figure 17 for the plotted substrate degradation). The **xylose degradation** behavior of the batch- and the feed-part nearly had the same slope, the second run was a little bit slower after the addition of xylose and arabinose. While the first run of the batch 30 g/L xylose were degraded in 123 hours with a rate of 0,24 g/L/h, in the second run 28 g/L xylose were degraded in 114 hours with a rate of 0,25 g/L/h.

At the first run xylose and arabinose feed was added at 123,5 hours and in the second run at 114 hours. The fed xylose in the first run was degraded with a rate of 0,27 g/L/h (90,5 g/L xylose in

333 hours), while in the second run it was degraded with a rate of 0,21 g/L/h (70 g/L xylose in 333 hours). The xylose degradation in the second run was 22% slower than the first run and therefore xylose was not yet degraded, when the fermentation was stopped. A possible reason for the slower degradation is the clogged sparger (occurred around 299 hours), which reduced the introduction of the air into the fermentation broth.

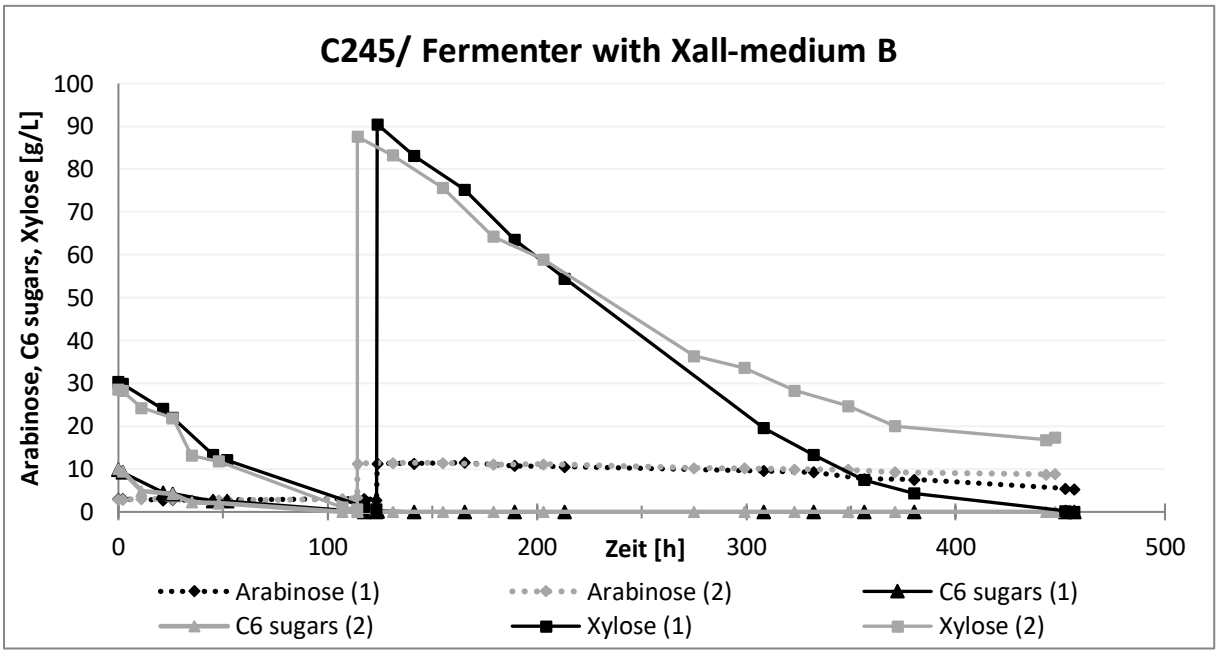

Figure 17: Substrate concentration of strain C245 with Xall-medium B

Arabinose degradation in these runs was low. In the first run the arabinose concentration in the feed-part was reduced by 53% and in the second run by 21%. In the second run the arabinose degradation in the feed-part (from 280 to 450 hours fermentation time) was slower, which could be a reason of the higher xylose concentration (discussed in chapter 5.2.1.4).

The two yeast products **ethanol and xylitol**, are plotted over fermentation time in Figure 18. The drop in the product concentrations at 114 and 123,5 hours marks the addition of the sugar feed and the dilution of the fermentation broth.

In both runs, only a small amount of ethanol concentration was produced (see Figure 18 and Figure 19). In the first run ethanol reached a maximum concentration of 1,4 g/L and in the second run 1,5 g/L. The ethanol concentration reached the maximum at the beginning of the batch-part of the fermentations (around 35-45 hours), when the C6 sugars were degraded. Afterwards the ethanol concentration decreased. This means that the yeasts produced ethanol from the C6 sugars only. After all hexoses were degraded, the yeasts started to consume the ethanol.

While the strain C245 only produced small amount of ethanol, it produced a high concentration of xylitol. In the first run a maximum xylitol concentration of 73,7 g/L from 115,8 g/L xylose in 380 hours was formed. In the second run 66,8 g/L xylitol was formed in 448 hours with 99,5 g/L xylose. When the xylose concentration in the first run was less than 4 g/L, the xylitol concentration decreased slowly. Perhaps the yeast started to consume the xylitol (similar to the first run of C238 in chapter 5.2.1.2).

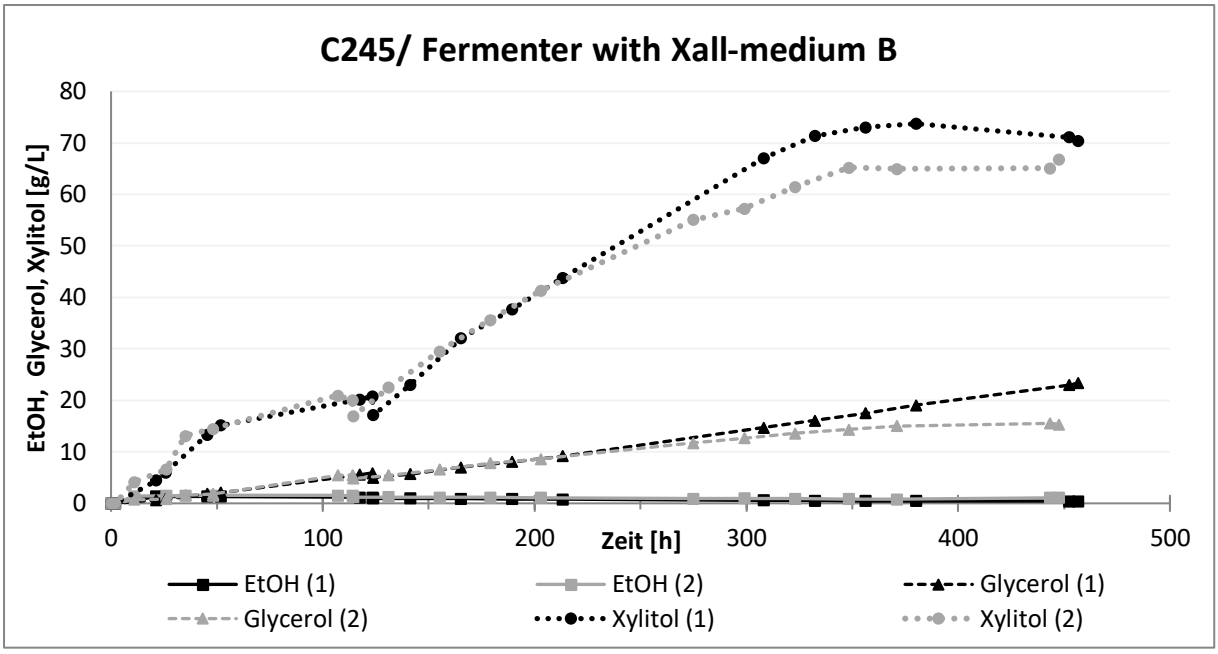

Figure 18: Main products of strain A027 with Xall-medium B. Intended products were ethanol & xylitol.

The **concentration of the side product glycerol** was also plotted in Figure 18, because it reached a high concentration in both fermentation runs. The glycerol concentration increased linearly over the fermentation time. In the first run the maximum glycerol concentration was 23 g/L at 457 hours and in the second run 15,5 g/L at 443 hours.

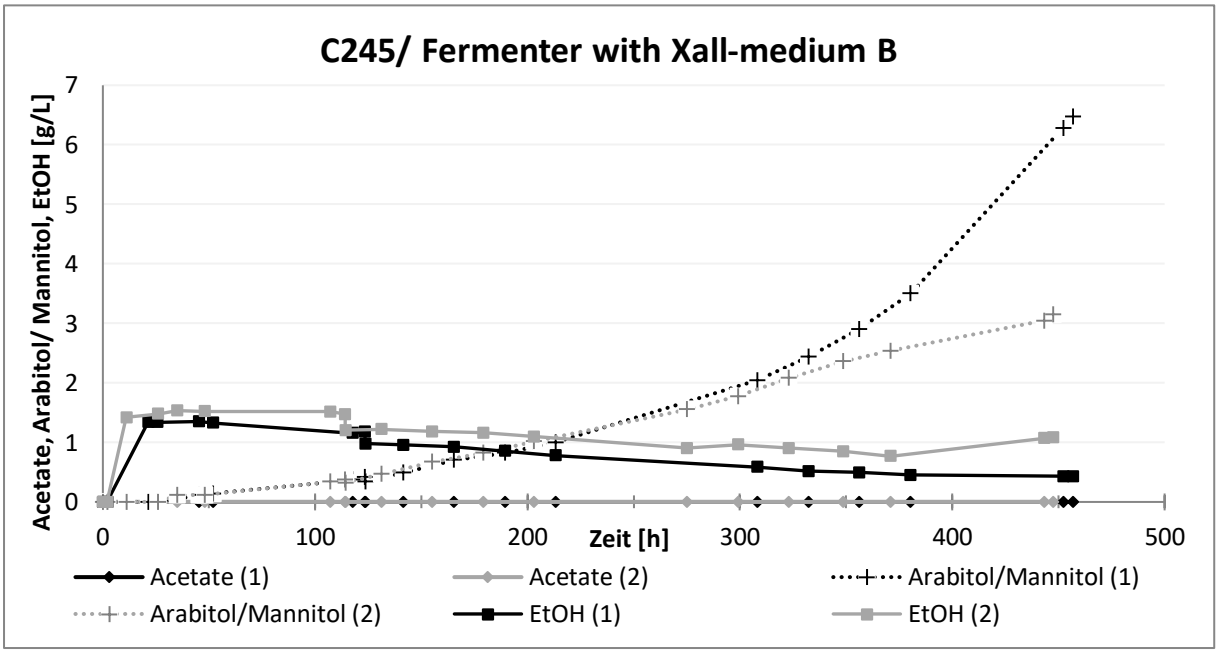

Figure 19: Side products of strain C245 with Xall-medium B. Ethanol was inserted again in order to demonstrate the low concentration.

Figure 19 also shows the **side products acetate and arabitol/mannitol** of C245 in Xall-medium B. In both runs no acetic acid was formed, but arabitol/mannitol reached a higher concentration in the end (run 1: 6,5 g/L, run 2: 3,2 g/L) than the ethanol concentration. The final arabitol/mannitol concentration of the first run was twice as high as the final concentration in the second run. A possible explanation could be the good degradation of xylose and consequently the better degradation of

arabinose in the first run (53% first run, 21% second run). For this reason, the detected arabitol/mannitol peak is probably arabitol, which was formed from arabinose.

5.2.1.4 Comparison of the fed-batch fermenters runs with Xall-medium

Table 36 to Table 45 show the batch- and feed-part results of the yeast strains which were tested in two consecutive runs in 1 L fermenters with Xall-medium B. The experiments were run until the xylose was nearly total degraded or earlier, in case of a slow degradation. For this reason, the duration of the experiments is given in most of the tables.

An overview of the **sugar consumption, the xylose and arabinose degradation rate** of the strains in the fermenters is shown in Table 36 to Table 38. The C6 sugars (glucose, galactose and mannose) were only present in the medium in the batch-part of the process. While A027 fully degraded the C6 sugars in 17 hours, C238 needed 20-40 hours and C245 140 hours.

Table 36: Selected yeast strains in the fermenter with Xall-medium B in a fed-batch process. Overview of the percentage of consumed xylose, the xylose degradation rates needed time for C6 sugar degradation.

Yeast strain	Process	Δ Run duration [h]	Δ Xylose End [%]	Δ Xylose [g/L/h]	Mean Δ Xylose [g/L/h]	100% Glucose degraded in _ hours
A027	Batch	324,8	86,1	0,08	0,08	17
		358,5	89,7	0,07		17
	Feed	308,8	12,6	0,04	0,06	-
		187,5	13,7	0,07		-
C238	Batch	193,3	99,2	0,15	0,17	40
		148,0	97,2	0,19		20
	Feed	279,8	99,5	0,33	0,27	-
		327,0	78,6	0,21		-
C245	Batch	123,3	97,8	0,24	0,25	140
		114,0	97,8	0,25		140
	Feed	333,5	100,0	0,27	0,24	-
		333,5	80,1	0,21		-

C238 and C245 degraded more than 97% of the xylose in the batch-phase and feed-phase in the first run and in the batch-phase of the second run. The second runs of both yeast strains were stopped before all xylose was degraded. The fermentations with A027 were stopped before all xylose was degraded, because of the slow degradation rate. The mean xylose degradation was 88% for the batch-phase and 13% for the feed-phase of the runs.

The fastest mean xylose degradation rate (see Table 36) of 0,27 g/L/h was achieved by the yeast strain C238 in the feed-phase of the fermentation. While in the batch-phase C238 only achieved a rate of 0,17 g xylose/L/h. A high xylose degradation rate was also reached by the yeast strain C245: in the batch-phase 0,25 g/L/h and in the feed-phase 0,24 g/L/h. A027 had the lowest degradation rate of the yeast strains, with 0,08 g/L/h in the batch and 0,06 g/L/h in the feed-phase.

Table 37 shows the xylose degradation rates in presence of C6 sugars, a high xylose concentration and a low xylose concentration. For the yeast strain A027, the xylose degradation rate was already low

and got lower by the end of the batch at a lower xylose concentration, but also after the addition of the sugar-feed.

For C238 and C245 it was different. In the batch-part, the xylose degradation rate was the highest in the presence of C6 sugars. The highest degradation rates of C238 and C245 were reached in the feed-part with a high xylose concentration, but without C6 sugars. The highest degradation of all yeast strains was reached in the feed-part of the first run of C238 with a value of 0,67 g/L/h, while the second run only reached 0,29 g/L/h. It seems like the speed of the degradation is not only dependent of the high CDM, but also from the fermentation time and the xylose concentration in the fermenter.

The second highest xylose degradation rate was calculated for C245 in the feed-part (run 1: 0,38 g/L/h and run 2: 0,32 g/L/h), also without C6 sugars, but at a high xylose concentration. The presence of C6 sugars seem to have no significant effect on the xylose degradation rate, but a higher xylose concentration appears to be encouraging for the degradation of xylose.

Table 37: Selected yeast strains in the fermenter with Xall-medium B in a fed-batch process. Overview of the percentage of the xylose degradation rate with C6 sugars, without C6 sugars and with low xylose concentration at the end of the fermentation.

Yeast strain	Process	Δ Run duration [h]	Δ Xylose with C6 sugars [g/L/h]	Δ Xylose without C6 sugars [g/L/h]	Δ Xylose without C6 & low xylose conc. [g/L/h]
A027	Batch	324,8	0,11	0,11	0,04
		358,5	0,06	0,09	0,04
	Feed	308,8	-	0,04	-
		187,5	-	0,07	-
C238	Batch	193,3	0,17	0,16	0,09
		148,0	0,23	0,18	0,14
	Feed	279,8	-	0,67	0,18
		327,0	-	0,29	0,15
C245	Batch	123,3	0,25	0,09	-
		114,0	0,26	0,06	-
	Feed	333,5	-	0,38	0,13
		333,5	-	0,32	0,11

The **arabinose degradation** (see Table 38) of all tested yeast strains was poor and too slow for an economically usage. The highest mean arabinose degradation rates were measured in the feed-part of the fermentation with C238 (0,010 g/L/h) and C245 (0,013 g/L/h). In the batch-part the mean arabinose degradation rate was for C238 0,007 g/L/h and for C245 0,008 g/L/h. The arabinose degradation rate of A027 was the lowest of the tested yeast strains, with 0,004 g/L/h in the batch and 0,003 g/L/h in the feed-part.

Table 38 shows, that there are no differences in the degradation rates of arabinose in presence of C6 sugars and xylose and with xylose only. It can be noticed, that the arabinose degradation rate with xylose in the feed-part of C238 and C245 was higher, than in the rest of the fermentations.

Table 38: Selected yeast strains in the fermenter with Xall-medium B in a fed-batch process. Overview of the percentage of consumed arabinose and the arabinose degradation rate at the end of the experiment, with C6 sugars and xylose and only with xylose.

Yeast strain	Process	Δ Run duration [h]	Δ Arabinose End [%]	Δ Arabinose [g/L/h]	Mean Δ Arabinose [g/L/h]	Δ Arabinose with C6+Xylose [g/L/h]	Δ Arabinose with Xylose [g/L/h]
A027	Batch	324,8	46,9	0,004	0,004	0,013	0,004
		358,5	37,3	0,003		0,002	0,003
	Feed	308,8	5,3	0,002	0,003	-	0,002
		187,5	4,8	0,003		-	0,003
C238	Batch	193,3	41,9	0,007	0,007	0,003	0,009
		148,0	33,0	0,007		0,013	0,006
	Feed	279,8	35,0	0,014	0,010	-	0,014
		327,0	14,7	0,005		-	0,005
C245	Batch	123,3	11,0	0,003	0,008	0,000	0,060
		114,0	35,9	0,012		0,000	0,000
	Feed	333,5	52,8	0,018	0,013	-	0,018
		333,5	20,8	0,007		-	0,007

The measured **optical density (OD_{600}), the cell dry mass (CDM) and the calculated biomass yield** (see chapter 4.4.9.4) of the yeast strains in the fermenter is summarized in Table 39.

The highest optical density was reached by C238 in the feed-part of the fermentation. The measured OD in the feed varies between 32 and 17 OD, presumably of aeration problems in the fermentation. The difference of the OD in the feed-part, can also be seen in the biomass yield which was 0,07 g CDM/g sugar in the first and 0,04 g CDM/g sugar in the second run.

Table 39: Selected yeast strains in the fermenter with Xall-medium B in a fed-batch process. Overview of the OD_{600}, CDM [g/L/OD], CDM at the end of the cultivation, CDM difference formed in the batch/feed-part and the biomass yield. Low biomass yield is marked green and higher one is marked red.

Yeast strain	Process	Δ Run duration [h]	OD_{600} End	ΔOD_{600} End	CDM [g/L/OD]	CDM End [g/L]	Δ CDM End [g/L]	Biomass yield [g CDM/g sugar]
A027	Batch	324,75	15,05	15,05	0,21	3,20	3,20	**0,09**
		358,5	14,28	14,28	0,22	3,18	3,18	**0,09**
	Feed	308,75	12,61	0,50	0,19	2,37	0,09	**0,01**
		187,5	14,11	2,42	0,19	2,71	0,46	**0,03**
C238	Batch	193,25	10,13	10,13	0,27	2,74	2,74	**0,07**
		148	10,30	10,30	0,22	2,30	2,30	**0,06**
	Feed	279,75	31,90	24,48	0,27	8,63	6,62	**0,07**
		327	16,74	8,22	0,30	5,07	2,49	**0,04**
C245	Batch	123,25	4,23	4,23	0,46	1,93	1,93	**0,05**
		114	4,78	4,78	0,26	1,23	1,23	**0,03**
	Feed	333,5	7,66	3,27	0,31	2,41	1,03	**0,01**
		333,5	7,21	2,67	0,28	2,05	0,76	**0,01**

To find out the mean OD and the reason of the deviation in the feed-part, the two fermentation runs with C238 should be repeated, which was not possible in this work for the lack of time. In the batch, C238 only reached 10 OD in both runs with a biomass yield of 0,06-0,07 g CDM/g sugar.

The second highest OD (mean 14,7 OD) with the highest biomass yield of 0,09 g CDM/g sugar, was measured for A027 in the batch-part. While the OD was constant (mean OD 13,4) after the dilution by the feed addition, the biomass yield was also low (0,01-0,03 g CDM/g sugar).

The lowest OD was measured for the yeast strain C245, with a mean optical density of 4,5 in the batch and 7,4 OD in the feed-part. C245 had the lowest biomass yield of the tested yeasts in the batch-part (0,03-0,05 g CDM/g sugar) and in the feed-part (0,01 g CDM/g sugar).

While the CDM per OD of A027 was between 0,19-0,22 g/L/OD and of C238 was between 0,22-0,30 g/L/OD, the CDM of C245 varied between the two fermentation runs. While the yeast strain C245 had between 0,26-0,28 g/L/OD in the first run in the batch- and feed-part, it had 0,46 g/L/OD in the batch-part of the first run and 0,31 g/L/OD in the feed-part. The deviation between the two runs could be a measurement error or a consequence of the aeration problem.

Table 40: Selected yeast strains in the fermenter with Xall-medium B in a fed-batch process. Overview of the calculated CDM yields: with C6+C5 sugars at the end and with C5 sugars between 0 g/L C6 and the END.

Yeast strain	Process	Δ Run duration [h]	CDM yield [g CDM /g sugar] END	CDM yield [g CDM/g C5] between 0g/L C6 and END
A027	Batch	324,75	0,089	0,054
		358,5	0,090	0,058
	Feed	308,75	0,007	
		187,5	0,033	
C238	Batch	193,25	0,065	0,042
		148	0,059	0,046
	Feed	279,75	0,069	
		327	0,035	
C245	Batch	123,25	0,048	-
		114	0,031	0,044
	Feed	333,5	0,011	
		333,5	0,010	

The highest CDM yields from all sugars until the end, were measured for the yeast strain A027 in the batch-part (0,089-0,090 g CDM/g sugar) and the lowest was measured for the feed-part of C245 (0,010-0,011 g CDM/g sugar). While the CDM yield for the C5 sugars in the culture bottles experiments (see Table 20 and Table 29) was supposed to be higher than the CDM yield from the C6 sugars, it was lower in the fermenter experiments with Xall-medium. Having a look on Table 40, the CDM yield from all sugars was higher, than the CDM yield from the C5 sugars in the batch-parts of A027 and C238. In the feed-parts the C6 sugars were already degraded, the CDM yield at the END therefore is equal to the CDM yield from C5 sugars. The CDM yields in the feed-parts were, except of the first run of C238, for all yeast strains lower than in the batch-parts of the Xall-medium fermenter experiments.

Table 41 shows the **pH decrease** (initial pH of 6 in the batch-phase) of the strains at the end of cultivation. Like in the pre-experiments in the culture bottles, the biggest pH decreases were registered

for A027 in the batch-part (1,45-1,68 pH units) and for C238 in the feed-part (1,41-2,39 pH units). The other fermentations had a lower decrease between 0,3-0,8 pH units. There was no correlation found between present organic acids.

Table 41: Selected yeast strains in the fermenter with Xall-medium B in a fed-batch process. Overview of the decrease of the pH at the end of the fermentation.

Yeast strain	Process	Δ Run duration [h]	Δ pH End
A027	Batch	324,75	-1,68
		358,5	-1,45
	Feed	308,75	-0,77
		187,5	-0,53
C238	Batch	193,25	-0,75
		148	-0,64
	Feed	279,75	-2,39
		327	-1,41
C245	Batch	123,25	-0,57
		114	-0,29
	Feed	333,5	-0,78
		333,5	-0,59

The maximum **ethanol and xylitol production** in the fermenter is summarized in Table 42 and Table 43. The tables also show the maximum yield of the products.

Table 42: Selected yeast strains in the fermenter with Xall-medium B in a fed-batch process. Maximum produced concentrations of the desired product ethanol in the batch- and feed-part of the fermentation, total maximum measured concentration of the respective part of the fermentation and calculated ethanol yield in the process part. Low max. ethanol concentration difference is marked red and higher one is marked green.

Yeast strain	Process	Δ Run duration [h]	Δ max. Ethanol [g/L]	Total max. Ethanol [g/L]	max. Ethanol yield [g EtOH /g sugar]
A027	Batch	324,75	**9,92**	9,92	0,28
		358,5	**10,30**	10,30	0,29
	Feed	308,75	**2,58**	10,87	0,25
		187,5	**3,46**	11,95	0,25
C238	Batch	193,25	**4,11**	4,11	0,10
		148	**4,26**	4,26	0,11
	Feed	279,75	**12,42**	15,98	0,13
		327	**5,88**	9,51	0,08
C245	Batch	123,25	**1,35**	1,35	0,05
		114	**1,53**	1,53	0,06
	Feed	333,5	**0,00**	0,98	-
		333,5	**0,01**	1,22	-

The highest ethanol concentration of 15,98 g/L (diluted ethanol from batch+ formed ethanol in the feed 12,42 g/L) was reached by C238 in the feed-part of the first run, while in the second run the maximum concentration was 9,51 g/L (diluted ethanol from batch+ 5,88 g/L formed in the feed-part). In the batch-part, C238 only produced a mean ethanol concentration of 4,2 g/L. C238 formed ethanol from hexoses and xylose (see chapter 5.2.1.2). C238 formed ethanol in the batch- and feed-part with an inefficient yield between 0,08-0,13 g ethanol/g sugar, compared to the xylitol yield of 0,40-

0,57 g xylitol/g xylose. C238 formed the second highest maximum xylitol concentration of 50 g/L in the first run and 52 g/L in the second run with a mean xylitol difference of 15,7 g/L in the batch and 38,0 g/L in the feed-phase (see Table 43).

While C238 produced the most ethanol in the feeding part of the process, the results were opposite for A027. A027 produced a mean ethanol concentration of 10,1 g/L in the batch-part and only 2,6-3,5 g/L in the feed-part (measured mean maximum ethanol concentration 11,41 g/L). Therefore, A027 had the highest ethanol yield of all tested strains with 0,28 g ethanol/g sugar in the batch and 0,25 g ethanol/g sugar in the feed-part. A027 converted xylose to ethanol, but with a slower formation rate (see chapter 5.2.1.1), because of the dependence of the slow xylose degradation rate. A027 also produced small amount of xylitol (see Table 43), while the formed amount and the xylitol yield (0,11 g xylitol/g xylose) in the batch phase was higher, than in the feed-phase (0,02 g xylitol/g xylose).

The least amount of ethanol was formed by C245 (mean 1,44 g/L in the batch-part). As described in chapter 5.2.1.3, the yeast strain C245 merely produced ethanol during the degradation of C6 sugars. This can also be seen in the feed-part (see Table 42) of the fermentation runs, where no C6 sugars where added and the ethanol concentration difference was zero. C245 slowly degraded a part of the produced ethanol during the feed-part of the fermentation.

Table 43: Selected yeast strains in the fermenter with Xall-medium B in a fed-batch process. Maximum produced concentrations of the desired product xylitol in the batch- and feed-part of the fermentation, total maximum measured concentration at the end of the respective part of the fermentation and calculated xylitol yield in the process part. Low max. xylitol concentration difference is marked red and higher one is marked green.

Yeast strain	Process	Δ Run duration [h]	Δ max. Xylitol [g/L]	Total max. Xylitol [g/L]	max. Xylitol yield [g Xylitol /g Xylose]
A027	**Batch**	324,75	**2,35**	2,35	0,12
		358,5	**2,20**	2,20	0,09
	Feed	308,75	**0,06**	1,93	0,01
		187,5	**0,17**	1,97	0,02
C238	**Batch**	193,25	**15,59**	15,59	0,54
		148	**15,86**	15,86	0,57
	Feed	279,75	**36,94**	49,96	0,40
		327	**38,90**	52,06	0,57
C245	**Batch**	123,25	**20,77**	20,77	0,70
		114	**20,94**	20,94	0,76
	Feed	333,5	**56,58**	73,73	0,66
		333,5	**49,84**	66,75	0,71

C245 produced the highest xylitol concentration of 70,24 g/L in the feed-part (run 1: 73,73 g/L and run 2: 66,75 g/L). The concentration is composed of a mean maximum xylitol difference of 20,86 g/L in the batch and 53,21 g/L xylitol formation in the feed-part. The xylitol formation rate of C245 was the highest of the three yeast strains tested in the fermenter with Xall B. The formation yield was between 0,66-0,76 g xylitol/g xylose in the batch- and feed-part.

Since xylitol in this case is only produced from xylose (see Formula 10 in chapter 4.4.9.3) and ethanol is produced from all sugars (hexoses and pentoses, see chapter 4.4.9.2)., more analyses are needed to tell which amount of ethanol is produced from hexoses and which from pentoses. In Table 44, the ethanol yields at the timepoint of the maximum ethanol concentration and at total hexose

degradation are listed. At 0 g/L hexose sugars, the ethanol yields are calculated from C6 sugars and C6 plus C5 sugars.

Having a look on the ethanol yield with C6 sugars at 0 g/L hexoses, it can be seen that the yield is never higher than the theoretical ethanol yield from C6 sugars of 0,51 g/g (see chapter 5.1.1. on page 45). This means, in the presence of C6 sugars none of the tested yeast strain produced ethanol from C5 sugars in the fermenter fed-batch process. Additionally, less xylose and arabinose were degraded in the beginning before the hexoses were fully degraded.

Table 44: Selected yeast strains in the fermenter with Xall-medium B in the batch-part of the fed-batch process. Ethanol yield calculated with total sugar consumption (at the time point of maximum ethanol concentration), with C6+C5 consumption and C6 consumption (both at 0 g/L C6 sugars) and the ethanol yield of C5 sugars between 0 g/L and the maximum ethanol concentration.

Yeast strain	Ethanol yield [g EtOH /g sugar] MAX	Ethanol yield [g EtOH /g C6+C5 sugars] at C6 0 g/L	Ethanol yield [g EtOH /g C6 sugars] at C6 0 g/L	Ethanol yield [g EtOH /g C5] between 0g/L C6 and MAX
A027	0,28 0,29	0,37 0,36	0,43 0,41	0,23 0,26
C238	0,10 0,11	0,20 0,25	0,33 0,36	0,02 0,03
C245	0,05 0,06	0,03 0,04	0,12 0,15	- -

After the degradation of the C6 sugars, only the yeast strain A027 produced ethanol with a noticeable yield from the C5 sugars (0,23-0,26 g ethanol/g C5 sugars) in the batch and feed part. Similar to the experiments in the culture bottles, the xylose degradation rate was poor and the ethanol concentration was therefore too low. This means, none of the yeast strains produced ethanol from pentose sugars with a higher and economic yield.

Table 45: Selected yeast strains in the fermenter with Xall-medium B in a fed-batch process. Maximum produced concentration of ethanol and xylitol in the batch- and feed-part of the fermentation. Maximum produced concentrations acetic acid, glycerol and arabitol/mannitol.

Yeast strain	Process	Δ Run duration [h]	Δ max. Ethanol [g/L]	Δ max. Xylitol [g/L]	max. Acetic acid [g/L]	max. Glycerol [g/L]	max. Arabitol/ Mannitol [g/L]
A027	**Batch**	324,75 358,5	9,92 10,30	2,35 2,20	0,46 0,45	0,75 0,74	1,55 1,39
	Feed	308,75 187,5	2,58 3,46	0,06 0,17	2,46 0,36	0,86 1,00	1,59 1,56
C238	**Batch**	193,25 148	4,11 4,26	15,59 15,86	0,29 0,28	0,47 0,47	0,75 0,79
	Feed	279,75 327	12,42 5,88	36,94 38,90	0,00 0,00	1,93 1,78	4,66 2,72
C245	**Batch**	123,25 114	1,35 1,53	20,77 20,94	0,00 0,00	5,91 5,52	0,41 0,38
	Feed	333,5 333,5	0,00 0,01	56,58 49,84	0,00 0,00	23,38 15,53	6,47 3,15

In Table 45 the maximum **concentrations of the side products** are listed. The most important finding is the high amount of formed glycerol by C245 in the fed-batch runs. The maximum measured glycerol concentration of the first run was 23,4 g/L and the second run was 15,5 g/L. A027 and C238 only had a maximum concentration of glycerol in the batch and feed-part, which varies between 0,47 g/L to 2 g/L. The glycerol was accumulated over the whole fermentation process.

The maximum concentration of arabitol/ mannitol (same retention time in HPLC analysis, see chapter 4.4.5) varied between 0,4 g/L and 6,5 g/L. In both fermentation parts of A027 and the batch-parts of C238 and C245 had a maximum of 2 g/L. The formed arabitol/mannitol concentration in the feed-part of C238 (2,7-4,7 g/L) and of C245 (3,2-6,5 g/L) was higher. The concentration was for both yeasts higher in the first run, which could be related to the faster xylose degradation rate in the first runs of C238 and C245.

While C245 and C238 produced no or only a low concentration of acetic acid, a higher concentration of 2,5 g/L acetic acid was formed by A027 in the feed-part of the first run, but for the other parts of the runs the concentration was also below 0,5 g/L.

5.2.2 Hydrolysate in fed-batch with synthetic pentose sugars

After the experiments with synthetic medium (see chapter 5.2.1), the experiments with hydrolysate-medium (preparation see chapter 4.3.4) in the one-liter fermenter were carried out.

Since the production of hydrolysate was very time consuming, only the two most promising yeast strains were tested in the fermenter with hydrolysate-medium. The yeast strain C238 showed the highest ethanol concentration and the best xylose degradation rate in the culture bottles with hydrolysate-medium, while C245 showed the highest xylitol formation.

For the yeast strain C238, the experiments in the culture bottles with Xall-medium and hydrolysate-medium containing peptone & yeast extract had a positive effect on the fermentation. That is why the experiments with C238 in the fermenter with hydrolysate-medium were also carried out with peptone and yeast extract (hydrolysate-medium B). The hydrolysate-experiments of the yeast strain C245 in the test bottles showed that the product concentration was much higher without peptone & yeast extract (see chapter 5.1.2). Therefore, the experiments of C245 in the fermenter with hydrolysate were also done without these additives (hydrolysate-medium A). For lack of time, the experiments with Xall-medium could not be retried without peptone and yeast extract.

For the fed-batch process (see chapter 4.2.3.4.), at a certain xylose concentration a feed with xylose, arabinose and glucose was added to the culture broth. The aim was to achieve a total xylose concentration around 100 g/L and an arabinose and glucose concentration with the same ratio to the xylose like in the used hydrolysate.

5.2.2.1 C238/ unknown strain

The second fermenter run of the yeast strain C238 in the fermenter with hydrolysate-medium was highly contaminated with bacteria after the addition of xylose, arabinose and glucose.

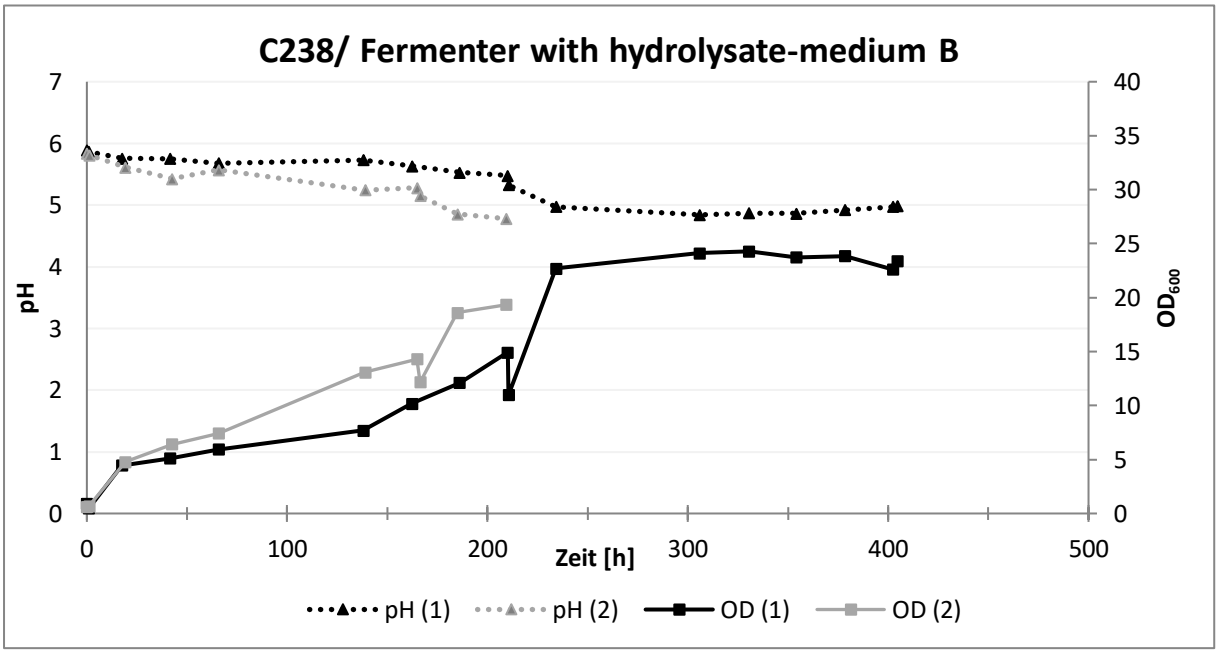

Figure 20: Measured optical density (OD) & pH-value of strain C238 with hydrolysate-medium B

The fermenter was stopped at 200 hours and just the data of the second run before the addition of the sugars (batch-part) can be used for evaluation. The experiment needs to be carried out again, but it was not possible to replicate the experiment for this work due to shortages of time and hydrolysate. In Figure 20 the measured **optical density (OD) and the pH-values** are plotted over fermentation time. Initially the OD-value was 0,5-0,7 and increased up to 14,3-14,9 OD with 28,3 g/L xylose and 8,7-10,9 g/L glucose. The OD increased until the sugar feed was added (run 1: 211 hours, run 2: 167 hours), were the fermentation broth was diluted. The OD decreased by the dilution to 11, but increased sharply in the next 24 hours to an OD of 22,7. The fast increase of the OD could be an effect of the high amount of added glucose (31 g/L in run 1). Until the end of the feed-part in run 1, the OD rose up to a value of 23,4 (at 404,5 hours; formed while 60 g/L xylose and 30,9 g/L glucose were degraded).

In the fermentation time of 404,5 hours, the pH-value decreased from an initial pH of 5,8 to a pH of 4,98 (run 1; only batch of run 2: pH 5,3 in 165 hours)

The curves with the **substrate concentrations** over the fermentation time are plotted in Figure 21. While the glucose, the only hexose in the hydrolysate, was degraded in 18 to 19,5 hours in the batch-part of the fermentation, the triple amount was degraded in the feed-part in less than 24 hours.

The **xylose degradation** behavior in the batch-parts of the runs nearly had the same slope. In the first run 28,3 g/L xylose were degraded in 210 hours with a rate of 0,13 g/L/h and in the second run 27,1 g/L xylose were degraded in 0,16 g/L/h. The degradation in the second batch run was therefore slightly faster.

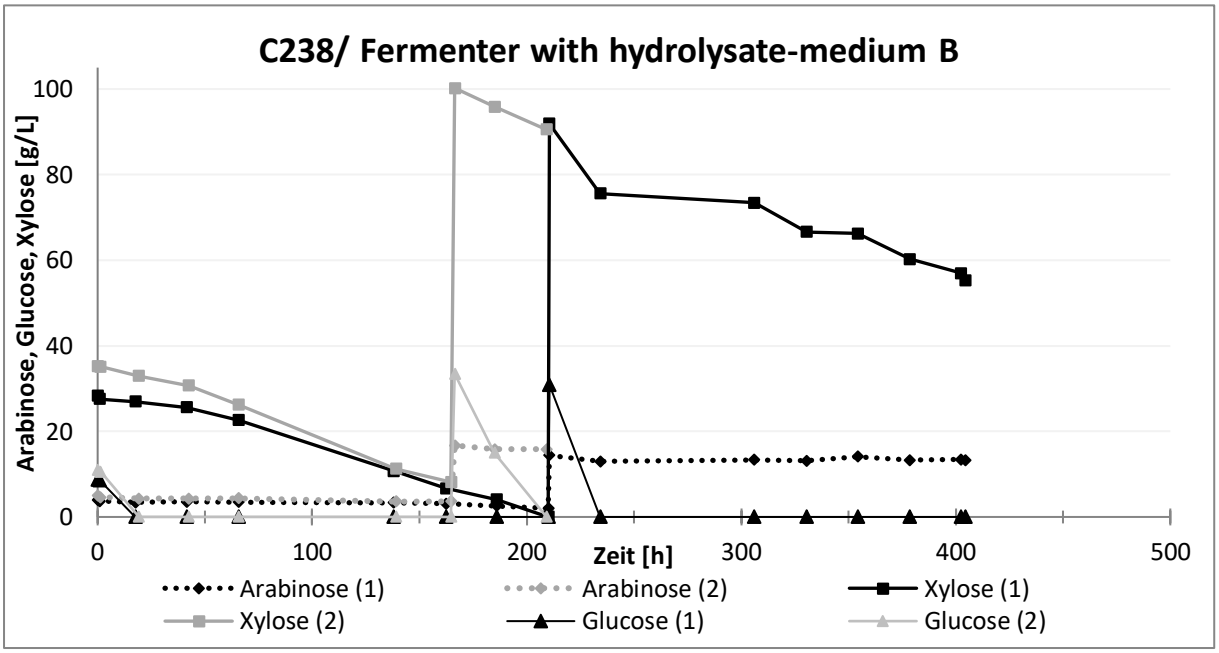

Figure 21: Substrate concentration of strain C238 with hydrolysate-medium B

In the first run the sugar feed with xylose, glucose and arabinose was added at 210 hours. Afterwards 36 g/L xylose were degraded in 194 hours. This results in a xylose degradation rate of 0,19 g/L/h in the feed-part of the first run. The data of the feed-part of the second run must be ignored, because of the contamination. Based on the data of the first run, the xylose degradation after the addition of the sugars was faster than in the batch-part. The reason could be the co-metabolism of

xylose with glucose, the higher xylose concentration or the increase of the biomass through the addition of a high amount of glucose (discussed in chapter 5.2.2.3). The comparison of the glucose and xylose curve in the beginning of the feed-part, shows that the xylose degradation rate is faster until the glucose is again fully degraded. This shows, that there must be a co-metabolism of the xylose with glucose, which enhances the xylose degradation.

The **arabinose concentration** was nearly constant over the fermentation time. In the first run in the batch-part 1,84 g/L were degraded in 210 hours (0,009 g/L/h) and in the feed-part 1,2 g/L in 194 hours (0,006 g/L/h). In the second run 1,3 g/L arabinose were degraded in 165 h (0,008 g/L/h). Based on the available data, the arabinose degradation in the batch-parts was slightly faster than in the feed-part of the first run. When the xylose concentration was lower, the arabinose degradation rate seems faster (further discussed in the comparison in chapter 5.2.2.3 in Table 49).

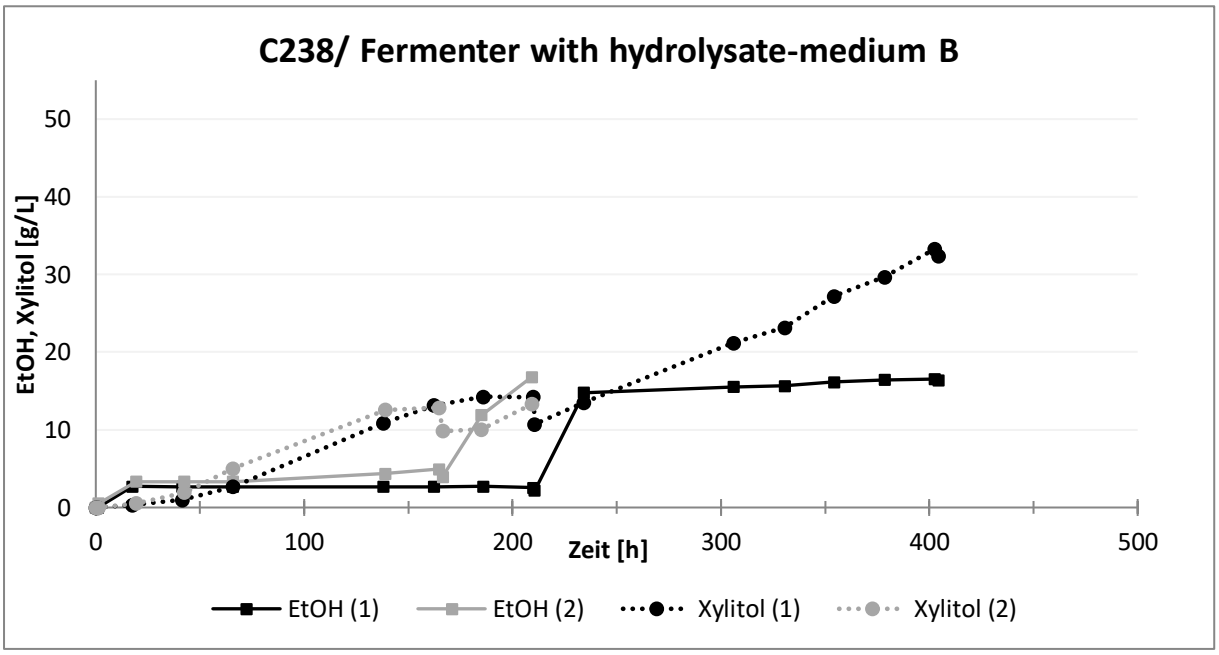

Figure 22: Intended products ethanol & xylitol of strain C238 with hydrolysate-medium B

The concentration of the main products, **ethanol and xylitol**, are plotted over fermentation time in Figure 22. The ethanol concentration for the batch-part of the first and second run was low. The maximum ethanol concentration in the batch-part of the first run was 2,7 g/L at 17,5 hours. In the second run the maximum ethanol concentration was 4,9 g/L in 165 hours. The ethanol concentration in the first run was therefore constant in the batch-part after the degradation of glucose, but the ethanol concentration still increased in the feed-part of the first run (by 1,73 g/L) and in the second run in the batch-part (by 1,60 g/L).

After the addition of the sugar feed, the ethanol concentration in the first run increased in presence of xylose, arabinose and glucose from 2,2 g/L to 14,8 g/L. When only the pentoses were present it rose from 14,8 g/L (at 234,3 hours) to the maximum ethanol concentration of 16,5 g/L (at 402,5 hours; regarding for dilution by the feed max. produced ethanol 16,9 g/L). The ethanol in this fermentation therefore seems to be nearly only formed from glucose and only a little amount was formed from xylose.

The formed xylitol of the yeast strain C238 was twice as much than the formed ethanol. In the batch-part the maximum xylitol concentration in the first run was 14,2 g/L (in 210 hours from 28,3 g/L xylose) and in the second run 12,9 g/L (in 165 hours from 27,1 g/L xylose). In the feed-part of the first run the maximum xylitol concentration of 33,2 g/L was reached in 402,5 hours (from 63,3 g/L xylose; undiluted 36,7 g/L xylitol). The fermentation had to be stopped earlier. It can be seen, that the xylitol formation had a rising trend, when the fermentation was stopped.

The main **side products** were glycerol and arabitol/mannitol (see Figure 23). There was no acetic acid formed, but acetate was already in the hydrolysate-medium from the beginning and was degraded by the yeasts over the fermentation.

The maximum glycerol concentration in the batch-part of the first run was 1,5 g/L at 186 hours, while in the second run a maximum of only 0,75 g/L was measured at 66 hours. After the addition of the sugar feed the glycerol concentration strongly increased in the first run and slowly increased afterwards to a maximum concentration of 4,1 g/L at 403 hours. It can be assumed that there is a correlation between the strong biomass formation or the ethanol formation which happened after the addition of the sugar feed.

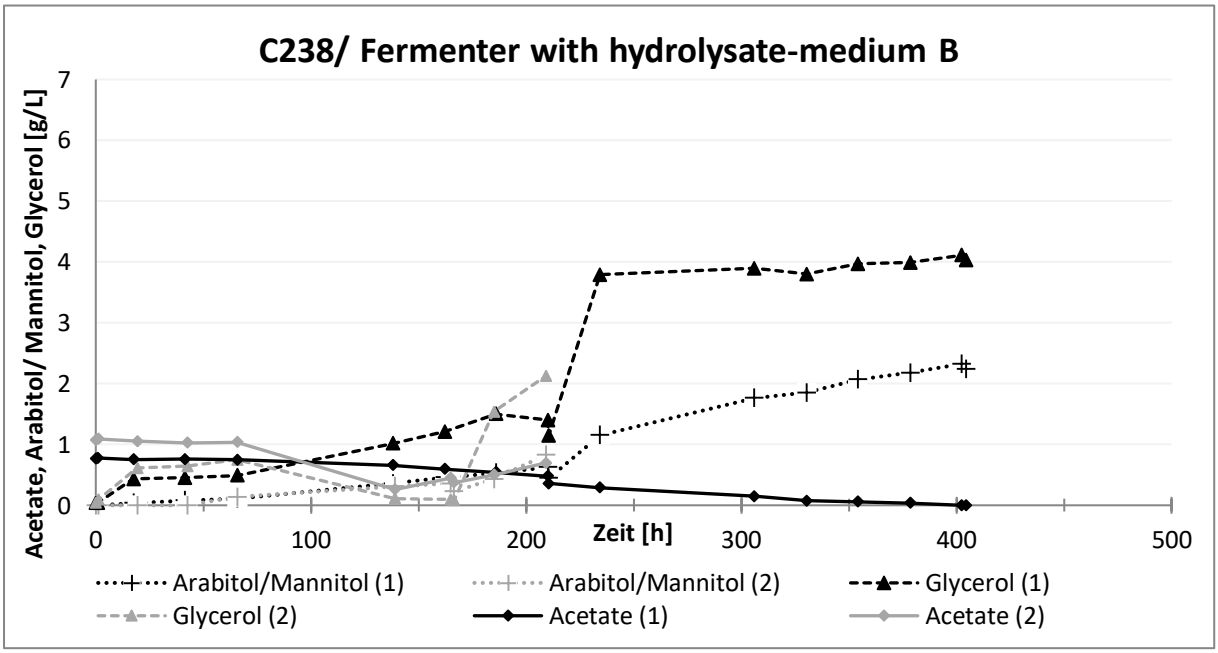

Figure 23: Side products of strain C238 with hydrolysate-medium B

The arabitol/ mannitol concentration of the first run reached a maximum of 2,3 g/L in the feed-part at 403 hours. In the data of the batch-part of the second run, only a maximum of 0,4 g/L was reached. Which is in the same range as the arabitol and mannitol concentration in the batch-part of the first run (0,6 g/L at 210 hours). In the curve in Figure 23 it can be seen, that arabitol/ mannitol rose over the whole fermentation time and increased stronger at the end of the fermentation. The arabinose concentration was nearly constant over the feed-part. Consequently, the mixed arabitol and mannitol peak, measured with HPLC 2, has a lower probability of being arabitol, because arabitol needs to be formed from arabinose.

5.2.2.2 C245/ *Candida* sp.

Figure 24 shows the **pH-value and optical density** over the fermentation time of the yeast strain C245 in hydrolysate-medium A. The OD curve differs a lot between the two runs, which can be explained by the strong foam formation over the fermentation time. A stable 2-centimeter-thick foam layer was formed in both runs and a lot of biomass stuck to the walls of the fermenter. In consequence of the inhomogeneous fermentation broth, the measurement of the optical density during the fermentation was inaccurate. The OD value at the end of the fermentation run was very high because the biomass that was stuck to the wall of the fermenter was washed down before the OD measurement. Nonetheless, the data of the pH-values, the substrate and product concentrations of the two runs in Figure 25 to Figure 27 show a good reproducibility.

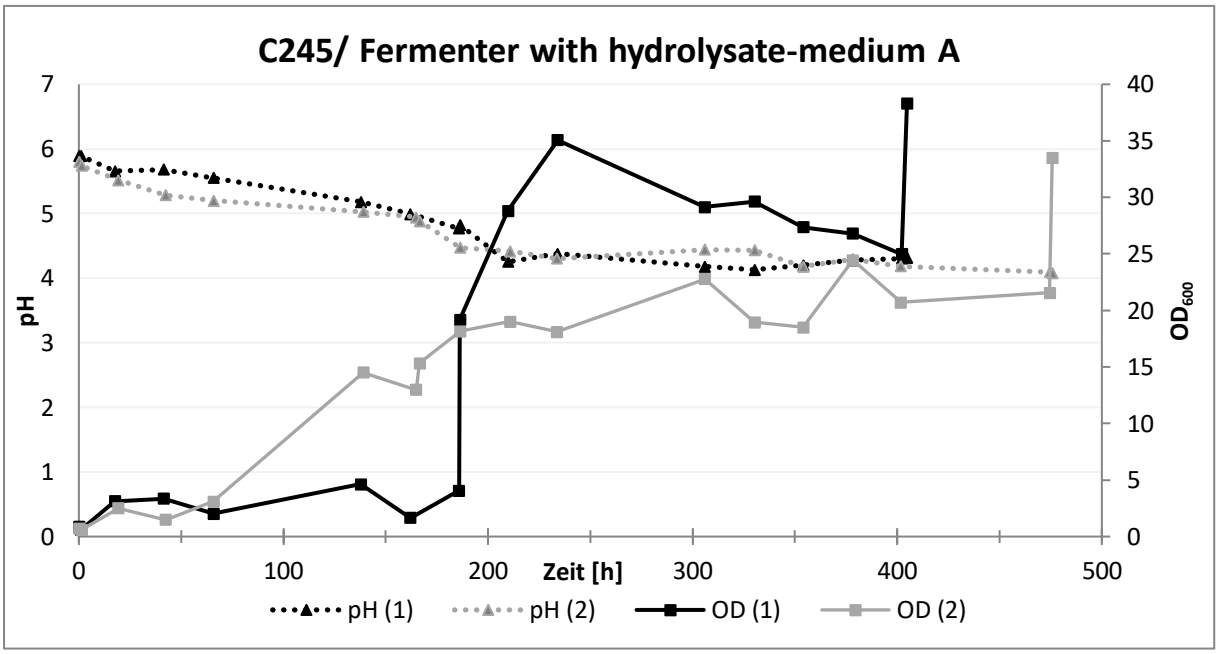

Figure 24: Measured optical density (OD) & pH-value of strain C245 with hydrolysate-medium A

The batch-part in run one ends at 186,3 hours and in the second run at 166,5 hours. The measured OD at the end of the batch-part in the first run was lower with 4,1 OD (from 25,7 g/L xylose and 9,1 g/L glucose) than the second run which had an OD between 13-14,5 OD (from 35,4 g/L xylose and 10,8 g/L glucose). The reason for the higher measured OD in the second run was the addition of antifoam-agent at 65,7 hours, when there was a risk of an overflow of the fermenter. Afterwards there was no more foam on the surface, means there must be a higher OD of the fermentation broth. When the sugar feed was added, the OD value in the second run increased only a little and fluctuated between 21 and 33,5 OD (last measured after rising of the biomass-film) with 128,91 g/L xylose and 44,0 g/L glucose. In contrast to this, the OD in the first run increased strongly (from 4,1 OD to 19,2 OD) after the addition of the sugar feed, because the antifoam-agent was added with the addition of the sugar feed (at 186 hours) and the biomass from the surface was suspended in the fermentation broth. Afterwards the OD was still increasing (with 107,41 g/L xylose and 40,5 g/L glucose) over the feed-part and fluctuates between 25,0 and 38,3 OD (last measured after rising of the biomass-film). At the end of the feed-part the OD was nearly in the same range in both fermentation runs.

While the OD curves varied widely, the pH of the two runs had a good comparability. The pH-value decreased from pH of 5,8 to pH of 4,3 in the first run and 4,1 in the second run.

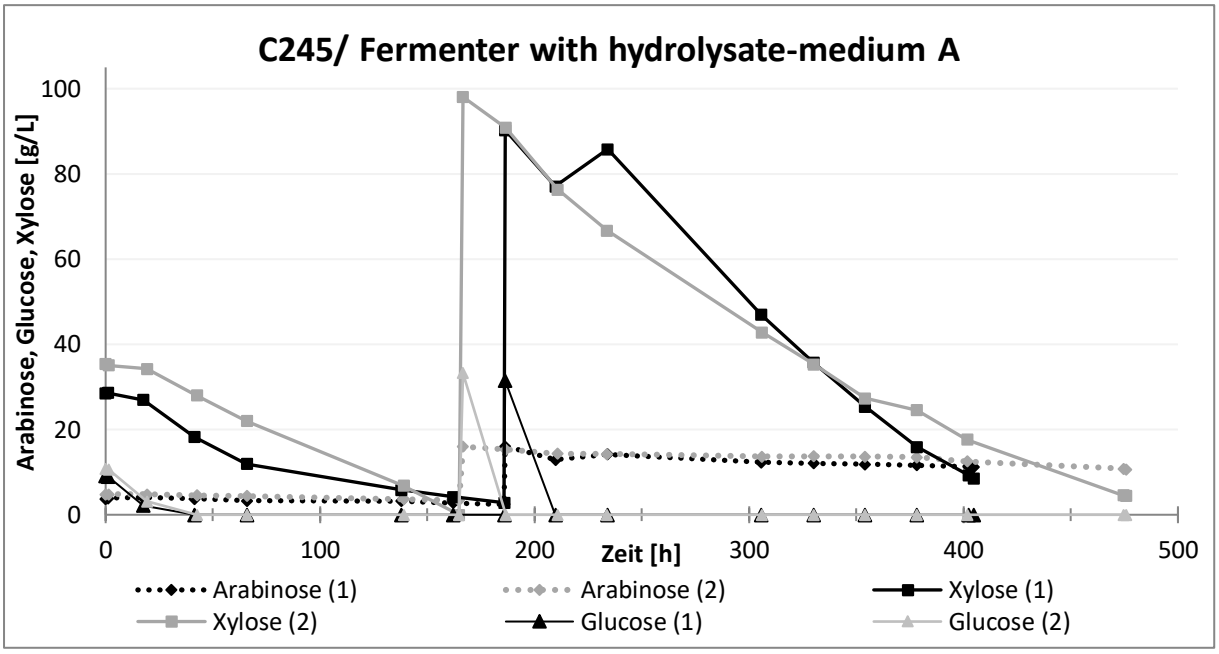

Figure 25: Substrate concentration of strain C245 with hydrolysate-medium A

The **substrate degradation** over the fermentation time is plotted in Figure 25. The glucose, the only hexoses in the hydrolysate-medium, was degraded in 41,5-42,5 hours in the batch-part and about the triple amount of glucose was degraded in the feed-part in 24 hours.

The **xylose degradation** behavior in the batch- and feed-part had similar slopes. The deviating measuring point in the first run at 234 hours must be a measurement failure of the HPLC 2. HPLC 1 determined 74 g/L xylose at 234 hours, which suites into the xylose-curve of the first run. In the batch-part of the first run, 25,7 g/L xylose were degraded in 186 hours, which gives a degradation rate of 0,14 g/L/h. While in the second run 35,4 g/L xylose were degraded in 165 hours. The xylose degradation rate in the second run is therefore 0,21 g/L/h and faster than in the first run.

The sugar feed with xylose, glucose and arabinose was added at 186,3 hours in the first and 166,5 hours in the second run. In the feed-part of the fermentation, 81,9 g/L xylose were degraded in 218,5 hours with a degradation rate of 0,37 g/L/h in the first run and in the second run 93,5 g/L xylose were degraded in 309,3 hours with a rate of 0,30 g/L/h. The degradation rate in both runs was faster in the feed-parts than in the batch. The reason could be similar like for the yeast strain C238: the co-metabolism of xylose with glucose, the higher xylose concentration or the increase of the biomass (see chapter 5.2.2.3, Table 47) through the addition of a high amount of glucose introduce a faster degradation rate in the feed-part. The comparing of the glucose and xylose curve at the beginning of the feed-part shows, that the xylose degradation rate is faster until the glucose is fully degraded. There must be a co-metabolism between glucose and xylose.

Based on the curve in Figure 25 the **arabinose concentration** was nearly constant over the fermentation time. In the batch-part of the first run 1,3 g/L arabinose were degraded in 186 hours (0,007 g/L/h), while in the second run the same quantity of arabinose was degraded in only 165 hours (0,008 g/L/h). The arabinose degradation was for both runs faster in the feed-part. In the

first run 4,85 g/L arabinose were degraded in 218,5 hours with a degradation rate of 0,022 g/L/h. In the feed-part of the second run 5,28 g/L arabinose were degraded in 308,3 hours with 0,017 g/L/h. When the xylose concentration was lower, the arabinose degradation rate seems faster (discussed in the comparison in chapter 5.2.2.3 in Table 49).

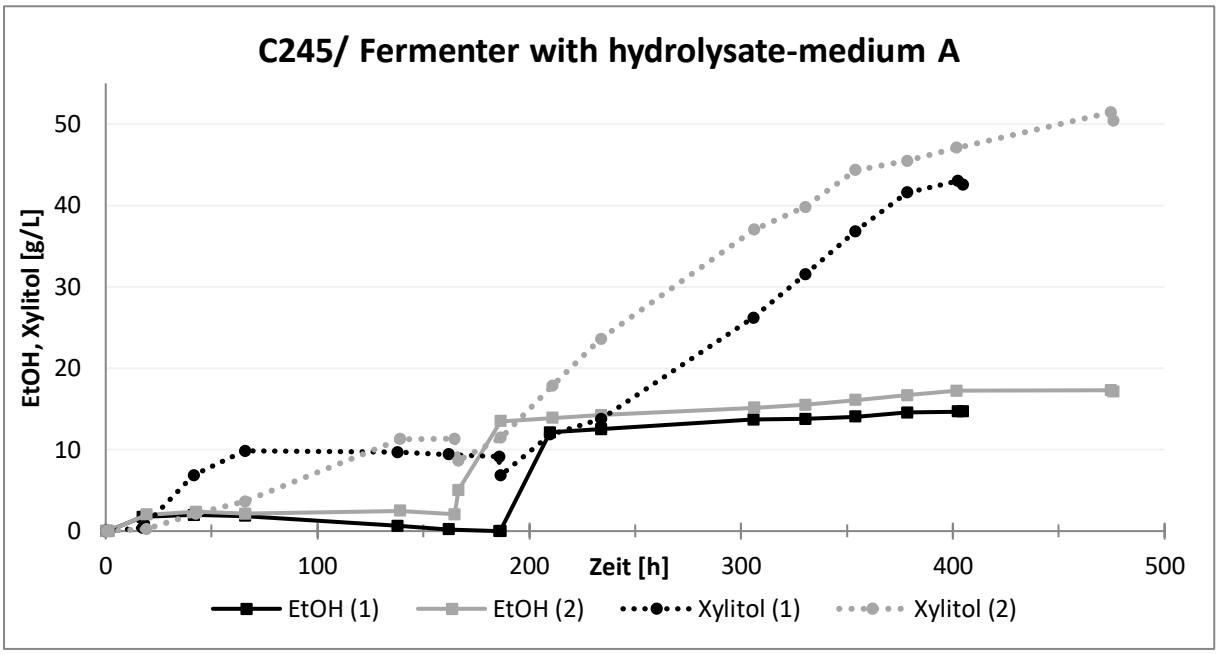

Figure 26: Intended products ethanol & xylitol of strain C245 with hydrolysate-medium A

Figure 26 shows the concentrations of the main **products ethanol and xylitol** over the fermentation time. First of all, the biggest part of the ethanol seems to be formed from glucose. In the batch-part of the first run the maximum ethanol concentration was 1,96 g/L (at 42,5 hours) and in the second run 2,5 g/L (at 139 hours). The ethanol formed in the batch was degraded, after the maximum was reached. The sugar feed was added at 186,3 hours in the first run and at 166,5 hours in the second run. The maximum ethanol concentration in the feed-part of the first run was 14,7 g/L (at 404,8 hours) and of the second run was 17,3 g/L (at 474,5 hours). In the first run only 2,6 g/L and in the second run 3,8 g/L of the ethanol were formed after the glucose of the sugar feed was already degraded. While in the feed-part in the presence of glucose 12,1 g/L ethanol in the first run and 13,5 g/L ethanol in the second run were formed. Yeast strain C245 also formed ethanol from the pentoses xylose and arabinose, but with a lower formation rate.

Xylitol was formed in a twice higher concentration than ethanol. The maximum xylitol concentration in the batch-part of the first run was 9,9 g/L at 65,8 hours from 16,6 g/L xylose. The xylitol concentration only decreased after this time point until the sugar feed was added. The second run was different, the maximum xylitol concentration of 11,3 g/L from 35,4 g/L xylose was reached at 165 hours. This means that the xylitol was formed over the whole batch-part. After the addition of the sugar feed, the xylitol concentration strongly increased over the whole fermentation time of both runs. The maximum xylitol concentration in the end of the feed-part of the first run was 43,0 g/L at 402,3 hours (regarding for dilution by the feed, max. produced 44,9 g/L xylitol from 106,6 g/L xylose) and in the second run 51,4 g/L xylitol in 474,5 h (undiluted 54,1 g/L xylitol from 128,9 g/L xylose). The

fermentation was stopped earlier, but it can be seen that the xylitol production had a rising trend at the end.

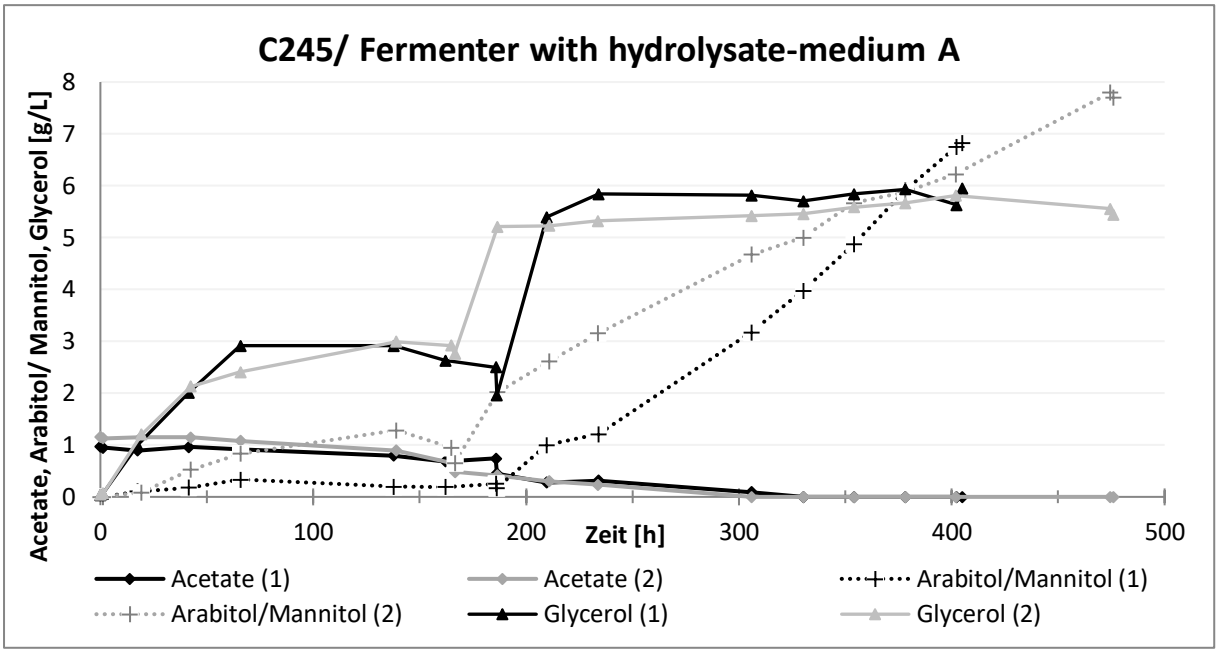

Figure 27: Side products of strain C245 with hydrolysate-medium A

Figure 27 shows the **concentration of the side products** in the fermentation of C245 with hydrolysate-medium. Acetate was initially in the hydrolysate-medium and was only degraded by the yeast cells over the fermentation. The main side products were therefore glycerol and arabitol/mannitol (measured as one peak in HPLC 2).

The maximum glycerol concentration in batch-parts of the runs was between 2,9-3 g/L (first run at 65,8 hours, second run at 139 hours). While the maximum glycerol increased in the feed-part up to a concentration of 5,8-5,9 g/L (first run at 378,3 hours, second run at 401,8 hours). The strongest increase of the glycerol concentration was right at the beginning of the batch- and feed-part. It seems like the glycerol formation is enhanced by the presence of glucose or of the formation of ethanol (discussion see chapter 6.3.3).

The arabitol/ mannitol concentration in the batch-part had a maximum of 0,3-1,3 g/L in the two runs (at the same time points where the glycerol had the maximum: first run at 65,8 hours, second run at 139 hours). In the feed-part a maximum of 6,8 g/L arabitol and mannitol was reached at 404,8 hours in the first run and 7,8 g/L at 474,5 hours in the second run. In the batch-part the arabitol and mannitol concentration rose to a maximum and was degraded afterwards. In the feed-part the concentration rose strongly over the fermentation time. Due to the strong degradation of arabinose in the feed-parts, it can be assumed that the measured peak at the same retention time is only arabitol.

5.2.2.3 Comparison of the fermenters runs with hydrolysate-medium

In this chapter, the fermentation runs of the yeast strains C238 and C245 with hydrolysate-medium in one liter fermenters are compared. The results of the batch- and feed-parts of the yeast strains are shown in Table 46 to Table 56. The experiments were run in the batch and feed-parts until the xylose in the hydrolysate-medium was nearly total degraded or earlier, in case of a slow degradation rate. Therefore, the duration of the experiments is given in the following tables. For C238 only the data of the feed-part of the first run is listed in the tables, because the second run was stopped after the batch-part, due to a bacterial contamination.

The **sugar consumption** and the degradation rates of the C5 sugars listed in Table 46 to Table 49. The only present C6 sugar in the hydrolysate-medium was glucose, which was present in the beginning of the batch-part and after the addition of the feed. While the yeast strain C238 fully degraded the glucose in 17,5-19,3 hours in the batch, C245 needed twice as long. In the feed-part both yeast strains needed around 20-23,8 hours. The faster degradation in the feed-part - despite of the around three times higher glucose concentration – might be the higher cell density at the beginning of the feed-part (see OD in Table 50).

Table 46: Yeast strains C238 with hydrolysate-medium B and C245 with hydrolysate-medium A in a fed-batch process in the fermenter. Overview of the percentage of consumed xylose, the xylose degradation rates and needed time for C6 sugar degradation.

Yeast strain	Process	Medium	Δ Run duration [h]	Δ Xylose End [%]	Δ Xylose [g/L/h]	100% Glucose degraded in _ hours
C238	Batch	Hydrolysate A	210,0	100,0	0,13	17,5
			164,8	76,9	0,16	19,3
	Feed		194,5	39,9	0,19	23,8
C245	Batch	Hydrolysate B	185,8	90,1	0,14	41,5
			164,8	100,0	0,21	42,5
	Feed		219,0	90,7	0,37	23,5
			311,0	95,4	0,30	20,0

Due to the different running times and concentrations of the pentoses in the batch and the feed-part, the percentage of degraded xylose and arabinose is inconclusive. For C238 the degraded xylose was around 76,9-100 % in the batch and 39,9 % in the feed-part, which was stopped earlier because of the slow xylose degradation. For the yeast strain C245 the degraded xylose was between 90,1 % and 100 % in the batch and feed-part.

A better value for the comparison is the degradation rate in g/L/h. The highest **xylose degradation rate** of 0,30-0,37 g/L/h was reached by C245 in the feed-parts of the two runs. In the batch-part the xylose degradation rate was much lower with 0,14 g/L/h in the first run and 0,21 g/L/h in the second run. A reason for the higher degradation rate in the feed-part might be the higher density of the cells, which was determined with the OD and the CDM (see Table 50). For C238 the xylose degradation rate achieved in the batch was between 0,13-0,16 g/L/h and in the feed-part of the first run 0,19 g/L/h. The xylose degradation rate of C238 was therefore in both fermentation parts lower than the rate of C245. Comparing the xylose degradation rates in [g/L/h] to the specific xylose degradation rates in [g/g CDM],

listed in Table 47, it becomes more explicit. The specific xylose degradation rates of the yeast strain C245 was more than twice as high than the rates of C238. For both yeast strains the spec. xylose degradation rate [g/g CDM] was significantly lower in the feed-part than in the batch-part. Table 47 is clearly indicating that the higher degradation rates [g/L/h] originates from the higher CDM in the feed part. Even though the specific rate was lower in the feed-part, the xylose degradation rate [g/L/h] for both yeast strains was higher in the feed-part than in the batch-part, due to the much higher CDM. On this way, a higher CDM can increase the xylose degradation.

Table 47: Xylose degradation rate per CDM [g/h/g CDM], specific in [g/g CDM] and [g/L/h] of the yeast strains C238 with hydrolysate-medium B and C245 with hydrolysate-medium A in a fed-batch process in the fermenter.

Yeast strain	Process	Δ Xylose [g/g CDM]	Δ Xylose [g/g CDM/h]	Δ Xylose [g/L/h]
C238	Batch	2,56	0,87	0,13
		2,11	0,19	0,16
	Feed	1,04	0,04	0,19
C245	Batch	7,95	1,57	0,14
		5,67	0,48	0,21
	Feed	2,11	0,07	0,37
		2,03	0,07	0,30

Table 48 shows, the xylose degradation rates in presence of C6 sugars, a high xylose concentration and a low xylose concentration. The yeast strain C238 in the batch-part degraded xylose faster without C6 sugars (mean 0,10 g/L/h with C6 sugars, mean 0,16 g/L/h without). While C245 in the batch-part had a slightly faster xylose degradation in presence of C6 sugars (mean 0,21 g/L/h), than without (mean 0,18 g/L/h).

Table 48: Yeast strains C238 with hydrolysate-medium B and C245 with hydrolysate-medium A in a fed-batch process in the fermenter. Overview of the percentage of the xylose degradation rate with C6 sugars, without C6 sugars and with low xylose concentration at the end of the fermentation.

Yeast strain	Process	Δ Run duration [h]	Δ Xylose with C6 sugars [g/L/h]	Δ Xylose without C6 sugars [g/L/h]	Δ Xylose without C6+ low degradation [g/L/h]
C238	Batch	210,0	0,08	0,14	0,14
		164,8	0,12	0,17	-
	Feed	194,5	0,67	0,10	-
C245	Batch	185,8	0,25	0,13	0,06
		164,8	0,17	0,22	0,26
	Feed	219,0	0,55	0,21	-
		311,0	0,33	0,21	0,04

The highest xylose degradation rates of both yeast strains were reached in the feed-part with a high xylose concentration and with C6 sugars (C238 with C6 sugar, with a value of 0,67 g/L/h and second highest xylose degradation rate of 0,55 g/L/h with C245 first run 0,33 g/L/h with C6 sugars and a high xylose concentration in the second run). The higher xylose degradation rate in the first run may be explained by the higher CDM. At the beginning of the feed-part, the first run had 10 OD more than the second one. The xylose degradation rate of C245 without C6 sugar but with a high xylose

concentration was similar in the feed-parts of the two runs (0,21 g/L/h), while it was significant lower for C238 (0,10 g/L/h).

In summary, the presence of C6 sugars in the hydrolysate-medium seem to have no significant effect on the xylose degradation rate. But as described in chapter 5.2.1.4, the higher xylose concentration at the beginning of the feed-part seems to enhance the yeasts to degrade the xylose.

Table 49: Yeast strains C238 with hydrolysate-medium B and C245 with hydrolysate-medium A in a fed-batch process in the fermenter. Overview of the percentage of consumed arabinose and the arabinose degradation rate at the end of the experiment, with C6 sugars and xylose and only with xylose.

Yeast strain	Process	Δ Run duration [h]	Δ Arabinose End [%]	Δ Arabinose [g/L/h]	Δ Arabinose with C6+Xylose [g/L/h]	Δ Arabinose with Xylose [g/L/h]
C238	Batch	210,0	47,3	0,009	0,027	0,007
		164,8	27,0	0,008	0,035	0,005
	Feed	194,5	8,3	0,006	0,057	0,003
C245	Batch	185,8	35,2	0,007	0,001	0,009
		164,8	27,8	0,008	0,004	0,009
	Feed	219,0	30,1	0,022	0,132	0,013
		311,0	33,1	0,017	0,029	0,012

Both yeast strains **degraded arabinose** too slow (see Table 49) for an economical use. The arabinose degradation rate over the fermentation of C238 was in the batch and feed-part between 0,006-0,009 g/L/h. In the batch-part the arabinose degradation was also in this range, but in the feed-part it increased up to a degradation rate of 0,017 g/L/h in the second run and 0,022 g/L/h in the first run. It seems, that the presence of glucose enhances the arabinose degradation rate of 238. But there was no clear tendency of the degradation rates of arabinose with C6 sugars+ xylose and the degradation rates only with xylose of the yeast strain C245.

Table 50: Yeast strains C238 with hydrolysate-medium B and C245 with hydrolysate-medium A in a fed-batch process in the fermenter. Overview of the OD600, CDM [g/L/OD], CDM at the end of the cultivation, CDM difference formed in the batch/feed-part and the biomass yield. Low biomass yield is marked green and higher one is marked red.

Yeast strain	Process	Δ Run duration [h]	OD_{600} End	CDM [g/L/OD]	CDM End [g/L]	Δ CDM End [g/L]	Biomass yield [g CDM/g sugar]
C238	Batch	210	14,93	0,28	4,17	4,17	**0,11**
		164,75	14,29	0,24	3,40	3,40	**0,09**
	Feed	194,5	23,36	0,25	5,73	3,04	**0,04**
C245	Batch	185,75	4,05	0,19	0,78	0,78	**0,02**
		164,75	12,97	0,25	3,28	3,28	**0,07**
	Feed	219	38,28	0,25	9,45	4,71	**0,04**
		311	33,51	0,28	9,47	5,14	**0,04**

The **optical density (OD_{600}), the cell dry mass (CDM) and the calculated biomass yield** (calculation see chapter 4.4.9.4) of the yeast strains C238 and C245 with hydrolysate-medium in the fermenter is summarized in Table 50. The highest optical density of 38,3 (first run) and 33,5 (second run) was reached by C245 in the feed-parts of the fermentations. The mean cell dry mass was therefore 9,5 g/L and the biomass yield 0,04 g CDM/g sugar in the feed-part of C245. The OD in the batch of C245 varied a lot between the two runs. The first run reached an OD of 4,1 (0,78 g/L) and the second run 13,0 OD (3,3 g/L CDM). The difference can also be seen in the biomass yield, which was 0,02 g CDM/g sugar in

the first and 0,07 g CDM/g sugar. This difference is a consequence of the biomass layer on the surface of the fermentation broth. The mean biomass yields of the two runs of the batch and the feed-part are therefore similar.

Table 51: Yeast strains C238 with hydrolysate-medium B and C245 with hydrolysate-medium A in a fed-batch process in the fermenter. Overview of the calculated CDM yields: with C6+C5 sugars at the end and with C5 sugars between 0 g/L C6 and the END.

Yeast strain	Process	Δ Run duration [h]	CDM yield [g CDM /g sugar] END	CDM yield [g CDM/g C5] between 0g/L C6 and END
C238	Batch	210	0,107	0,103
		164,75	0,086	0,089
	Feed	404,5	0,044	0,014
C245	Batch	185,75	0,021	0,008
		164,75	0,069	0,100
	Feed	404,75	0,040	0,056
		475,75	0,039	0,054

For the yeast strain C238 the two runs had a better comparability. While in the batch-part the first run had an OD of 14,9 (4,2 g/L CDM), the second run had an OD of 14,3 (3,4 g/L CDM). The reached OD in the feed-part of C238 was higher with 23,4 and a CDM of 5,7 g/L. The biomass yield of the batch of C238 was higher with a mean value of 0,1 g CDM/g sugar, while in the feed-part the biomass yield was the same as for C245 (0,04 g CDM/g sugar). The CDM per OD of C238 was between 0,24-0,28 g/L/OD and of C245 was between 0,19-0,28 g/L/OD.

The highest CDM yields were found for the batch-parts of C238 in hydrolysate-medium (0,086-0,107 g CDM/g sugar until end and 0,089-0,103 g CDM/g C5 sugars between 0 g/L C6 and the end).

Other than in the experiments in the culture bottles (see chapter 5.1) and in the fermenter with Xall-medium (see chapter 5.2.1.4, Table 40), no clear conclusion can be made from the calculated CDM yield of all sugars until the end and the CDM yield of C5 sugars.

Table 52: Yeast strains C238 with hydrolysate-medium B and C245 with hydrolysate-medium A in a fed-batch process in the fermenter. Overview of the decrease of the pH at the end of the fermentation.

Yeast strain	Process	Δ Run duration [h]	Δ pH End
C238	Batch	210	-0,41
		164,75	-0,55
	Feed	194,5	-0,33
C245	Batch	185,75	-1,12
		164,75	-0,78
	Feed	219	-0,51
		311	-0,80

The initial pH in the batch-phase was 6, the **pH decrease** at the end of the fermentation with the yeast strains is shown in Table 52. The maximum pH decrease was only 1,1 pH-units, of C245 in the batch-part of the first run. The other fermentation-parts had a lower decrease between 0,3-0,8 pH units. There was no correlation found between the pH decrease and the other data. The slight decrease

of the pH has the advantage, that it does not enhance the inhibitory effect of the acetic acid already included (see Table 56) in the hydrolysate-medium (Nigam, 2001).

The maximum concentrations and the yield of the most important **products ethanol and xylitol** is summed up in Table 53 and Table 55. The highest ethanol concentration of 17,30 g/L (diluted ethanol from batch+ formed ethanol in the feed 12,27 g/L) was reached by C245 in the feed-part of the second run. In the first run of C245 the maximum ethanol concentration in the feed-part was little bit lower at 14,70 g/L. Therefore, the maximum reached ethanol concentration of 16,53 g/L (diluted ethanol from batch+ 14,36 g/L formed in the feed-part) in the feed-part of C238 and the mean maximum ethanol concentration of 16,0 g/L in the feed-parts of C245 were in the same range. In the batch-parts C238 formed a mean maximum ethanol concentration of 3,82 g/L and C245 2,22 g/L.

Table 53: Yeast strains C238 with hydrolysate-medium B and C245 with hydrolysate-medium A in a fed-batch process in the fermenter. Maximum produced concentrations of the desired product ethanol in the batch- and feed-part of the fermentation, total maximum measured concentration of the respective part of the fermentation and calculated ethanol yield in the process part. Low max. ethanol concentration is marked red/yellow and higher one is marked green.

Yeast strain	Process	Medium	Δ Run duration [h]	Δ max. Ethanol [g/L]	max. Ethanol [g/L]	max. Ethanol yield [g EtOH /g sugar]
C238	Batch	Hydrolysate A	210,00	2,70	2,70	0,26
			164,75	4,93	4,93	0,13
	Feed		194,50	14,36	16,53	0,22
C245	Batch	Hydrolysate B	185,75	1,96	1,96	0,10
			164,75	2,48	2,48	0,06
	Feed		219,00	14,70	14,70	0,12
			311,00	12,27	17,30	0,09

The yeast strains C238 and C245 mostly produced ethanol from glucose only and a little amount from xylose with a lower formation rate (see chapter 5.2.2.1 and 5.2.2.2). The mean ethanol yield of C238 in the batch-part was 0,20 g ethanol/g sugar and 0,22 g ethanol/g sugar in the feed-part (see Table 53). For C245 the mean ethanol yield of the batch was 0,08 g ethanol/g sugar and of the feed-part was 0,11 g ethanol/g sugar. The ethanol yield of C238 was therefore twice as much as the ethanol yield of C245.

Table 54: Yeast strains C238 with hydrolysate-medium B and C245 with hydrolysate-medium A in a fed-batch process in the fermenter. Ethanol yield calculated with total sugar consumption (at the time point of maximum ethanol concentration), with C6+C5 consumption and C6 consumption (both at 0 g/L C6 sugars) and the ethanol yield of C5 sugars between 0 g/L and the maximum ethanol concentration.

Yeast strain	Process	Ethanol yield [g EtOH /g sugar] MAX	Ethanol yield [g EtOH /g C6+C5] at C6 0 g/L	Ethanol yield [g EtOH /g C6] at C6 0 g/L	Ethanol yield [g EtOH /g C5] between 0 g/L C6 and MAX
C238	Batch	0,26	0,26	0,31	-
		0,13	0,24	0,30	0,06
	Feed	0,22	0,30	0,48	0,09
C245	Batch	0,10	0,10	0,22	-
		0,06	0,13	0,22	0,01
	Feed	0,12	0,25	0,39	0,04
		0,09	0,33	0,41	0,04

Table 54 shows the calculated ethanol yield at the maximum ethanol concentration, when the hexoses are degraded completely and between these time points. The ethanol yield at 0 g/L glucose from C6 sugars is never higher than the theoretical ethanol yield from C6 sugars of 0,51 g/g (see chapter 5.1.1. on page 45), which means that in presence of glucose, no ethanol is formed of xylose or arabinose. The ethanol yield of the pentose sugars between the fully degradation of glucose and the maximum ethanol concentration was for both yeast strains too low for an economical usage.

Instead of ethanol, C245 and C238 formed xylitol with a higher yield (see Table 55). For C238 a maximum xylitol concentration of 14,24 g/L was measured in the first run of the batch and 12,87 g/L in the second run was measured. While in the feed-part a maximum of 33,24 g/L xylitol (diluted xylitol from batch+ formed xylitol in the feed 22,52 g/L) was reached. The mean xylitol yield for C238 was 0,53 g xylitol/g xylose in the batch-part and 0,64 g xylitol/g xylose in the feed-part.

Table 55: Yeast strains C238 with hydrolysate-medium B and C245 with hydrolysate-medium A in a fed-batch process in the fermenter. Maximum produced concentrations of the desired product xylitol, in the batch- and feed-part of the fermentation, total maximum measured concentration at the end of the respective part of the fermentation and calculated xylitol yield in the process part. Low max. xylitol concentration is marked red/yellow and higher one is marked green.

Yeast strain	Process	Δ Run duration [h]	Δ max. Xylitol [g/L]	max. Xylitol [g/L]	max. Xylitol yield [g Xylitol /g Xylose]
C238	Batch	210,00	**14,24**	**14,24**	0,59
		164,75	**12,87**	**12,87**	0,47
	Feed	194,50	**22,52**	**33,24**	0,64
C245	Batch	185,75	**9,85**	**9,85**	0,59
		164,75	**11,33**	**11,33**	0,32
	Feed	219,00	**36,20**	**42,99**	0,45
		311,00	**42,77**	**51,43**	0,46

C245 formed a maximum xylitol concentration of 9,85 g/L in the first run and 11,33 g/L in the second run of the batch. In the feed-part, C245 reached a maximum of 42,99 g/L xylitol in the first (diluted xylitol from batch+ formed xylitol in the feed 36,20 g/L) and the highest xylitol concentration of all strains of 51,43 g/L in the second run (diluted xylitol from batch+ formed xylitol in the feed 42,77 g/L). C245 formed xylitol with a mean yield of 0,46 g xylitol/g xylose in the batch and feed-part. In the previous experiments C245 was known as xylitol producing yeast strain and reached the highest xylitol concentration in the hydrolysate-medium in the fermenter experiments. Contrary to the expectations, the yeast strain C238 with the main purpose of ethanol production, had a 28 % higher xylitol yield than C245 in the fermentation with hydrolysate-medium.

The maximum **concentration of the side products** is listed in Table 56. The yeast strains produced no acetic acid with the hydrolysate-medium, the acetic acid was contained in the hydrolysate at the beginning. The yeast cells degraded the acetic acid over the fermentation runs. Measured side products were therefore glycerol and arabitol/mannitol (measured as one peak in HPLC 2).

Table 56: Yeast strains C238 with hydrolysate-medium B and C245 with hydrolysate-medium A in a fed-batch process in the fermenter. Maximum produced concentration of ethanol and xylitol in the batch- and feed-part of the fermentation. Maximum produced concentrations of glycerol and arabitol/mannitol. Acetic acid included in the hydrolysate-medium.

Yeast strain	Process	Medium	Δ max. Ethanol [g/L]	Δ max. Xylitol [g/L]	Initial Acetic acid [g/L]	max. Glycerol [g/L]	max. Arabitol/ Mannitol [g/L]
C238	Batch	Hydrolysate A	2,70	14,24	0,77	1,50	0,63
			4,93	12,87	1,07	0,75	0,36
	Feed	Hydrolysate A	14,36	22,52	0,36	4,11	2,32
C245	Batch	Hydrolysate B	1,96	9,85	0,97	2,91	0,34
			2,48	11,33	1,16	2,99	1,29
	Feed	Hydrolysate B	14,70	36,20	0,45	5,94	6,83
			12,27	42,77	0,48	5,81	7,79

The mean maximum glycerol concentration of C238 in the batch-part was 1,13 g/L, while for C245 it was more than twice as high with 2,95 g/L. The maximum glycerol concentration reached in the feed-part was 4,11 g/L for C238 and 5,88 g/L for C245. For both yeast strains, the glycerol concentration increased a lot at the beginning of the batch and feed-part in the presence of glucose and was nearly constant afterwards. There must be a correlation between the formation of glycerol and the glucose degradation or of the formation of ethanol (discussion see chapter 6.3.3).

The maximum concentration of arabitol together with mannitol (same retention time in HPLC analysis, see chapter 4.4.5) varies between 0,4 g/L and 7,8 g/L (see Table 56). For both yeast strains the arabitol/mannitol concentration was the highest at the end of the fed-batch parts. The maximum arabitol/mannitol concentration of C238 was 0,5 g/L for the batch and 2,32 g/L in the feed-part. The arabitol/mannitol concentration was hence lower than the glycerol concentration of C238 in both fed-batch parts. For the yeast strain C245 the maximum reached arabitol/mannitol concentration in the batch runs was 0,82 g/L. In the feed-part, the maximum arabitol/mannitol concentration was higher than the glycerol concentration, with a mean maximum concentration of 7,31 g/L. When the mean arabinose degradation rate in Table 49 is compared to the arabitol/mannitol concentration in the fermentation parts (see Table 56), the higher measured arabitol/mannitol concentration of C245 in the feed-part seems to correlate with the high arabinose degradation rate. Due to the stronger degradation of arabinose, the mixed peak is presumably arabitol.

5.2.3 Variations of fermentation runs of C245/ *Candida* sp.

Of the tested yeast strains for the production of xylitol, C245 is the most promising and hence additional experiments were carried out with this strain. To determine the effect of peptone and yeast extract in the fermenter, one run with Xall-medium A was carried out. Like described in chapter 3.5.2 a higher initial cell concentration can have a positive effect on the xylitol production from xylose (Winkelhausen and Kuzmanova, 1998). For this reason, an experiment was carried out with a 3-fold higher inoculum of 1,5 OD in Xall-medium A.

Due to limited time, no double determinations were carried out and the additional experiments should be repeated in Xall-medium and hydrolysate-medium. Nevertheless, the data showed interesting results which are succinctly explained in the following chapters 5.2.3.1 and 5.2.3.2. The tables with the full evaluated data is showed in the appendix chapter 9.5, on page 118.

5.2.3.1 C245/ Xall-medium A compared to Xall-medium B

The run with Xall-medium A showed less differences to the two runs with Xall-medium B. One interesting outcome is the much lower pH of 2,9 at the end with Xall-medium A, while with Xall-medium B was between pH 4,6-5. The same observation was made in the culture bottles (see chapter 5.1.1) and approves the possible buffering properties of peptone and yeast extract.

While the ethanol yield was similar, the reached maximum ethanol concertation in Xall-medium A was 0,5 g/L lower, due to the degradation of the ethanol once xylose is fully degraded. The concentration of xylitol and of the side products in Xall-medium A and B were nearly the same.

5.2.3.2 C245/ Xall-medium A with 3-fold inoculum

The increase of the initial optical density from OD 0,5 to 1,5 in Xall-medium A had a strong impact on the maximum optical density at the end of the fermentation. While an initial OD of 0,5 leads to a maximum OD of 6,2, the initial OD of 1,5 leads to a maximum OD of 4,8. The higher inoculum concentration also lead to less decrease of the pH-value (initial OD 0,5: 2,9; initial OD 1,5: 4,6).

The most relevant difference was reached for the xylose degradation. An initial OD of 0,5 leads to a xylose degradation rate of 0,20 g/L/h in the batch-part and 0,27 g/L/h in the feed-part. While an initial OD of 1,5 the xylose degradation was much faster with a rate of 0,34 g/L/h in the batch and 0,31 g/L/h in the feed-part.

The xylitol yield with the initial OD of 1,5 was with 0,82 g xylitol/g xylose in the batch-part much higher than with initial OD 0,5 (0,64 g xylitol/g xylose). In the feed-part the xylitol yield for the higher initial OD was 0,66 g xylitol/g xylose and lower than the run with lower initial OD (0,71 g xylitol/ g xylose). The maximum xylitol concentration of the run with the higher inoculum was therefore 1,8 g/L higher in the batch-part, but 3,5 g/L lower in the feed-part.

The ethanol concentration in the batch-part with the 3-fold higher inoculum was 0,3 g/L higher than with the lower inoculum and similar in the feed-parts. While the concentration of the side-product glycerol was similar in both runs, the arabitol/mannitol concentration was 50% lower in the run with the initial OD 1,5.

6 Discussion

In this chapter, the results of the pretests and fermenter runs are discussed. For the pretests in the culture bottles the yeast strains which were selected are described. In chapter 6.2, overall observations of the pretests and fermenter runs are discussed. Also, the experiments with the three favored yeast strains are compared and discussed, each yeast strain separately (see chapters 6.3). The results are compared with references and suggestions for future research are made.

6.1 Pretests - Limiting of the number of yeast strains

The purpose of the pretests was to limit the number of yeast strains for the fermenter experiments to the best ones. Three of the six yeast strains were already selected in the pretests.

In the experiments with Xall-medium, following yeast strains were selected:

HA1129/ *Candida intermedia (ATCC 201070)*: This yeast strain showed, even in synthetic Xall-medium, poor xylose and arabinose degradation properties (see Table 57). HA1129 was tested on ethanol formation, but it formed only a low ethanol concentration (below 3 g/L) from C6 sugars (see Table 58). The results contradict the results of the references, where *Candida intermedia* strains are described with a high capacity xylose transporter and their transporter genes are introduced into *S. cerevisiae* cells for a better xylose uptake rate (Gárdonyi et al., 2003, Leandro et al., 2006, Runquist et al., 2009, Fonseca et al., 2011). The reason for the discrepancy with the references can exist due to differences of the yeast strains or different media composition or cultivation conditions.

Table 57: HA1129 in the culture bottles with Xall-medium A and B. Overview of the percentage of consumed sugar at 305 hours and at the end of the experiment.

Yeast strain	Run duration [h]	Medium	Δ Xylose 305h [%]	Δ Xylose End [%]	Δ Arabinose 305h [%]	Δ Arabinose End [%]	Δ 100% C6 sugars at __ hours
HA1129	503,25	Xall A	28,5	38,0	11,0	14,8	114,5
		Xall B	18,1	28,9	7,1	13,0	114,5

Table 58: HA1129 in the culture bottles with Xall-medium A and B. Ethanol yield calculated with total sugar consumption (at the time point of maximum ethanol concentration), with C6+C5 and C6 consumption (both at 0 g/L C6 sugars) and the ethanol yield of C5 sugars between 0 g/L and the maximum ethanol concentration.

Yeast strain	Run duration [h]	Medium	Ethanol yield [g EtOH /g sugar] MAX	Ethanol yield [g EtOH /g C6+C5] at C6 0 g/L	Ethanol yield [g EtOH /g C6] at C6 0 g/L	Ethanol yield [g EtOH /g C5] between 0g/L C6 and MAX
HA1129	503,25	Xall A	0,22	0,25	0,36	0,01
		Xall B	0,28	0,28	0,40	-

C240/ *Candida utilis*: This yeast strain formed a low ethanol concentration around 3 g/L, but therefore formed a high xylitol concentration of 20 g/L and 16 g/L (see Table 59). The utilization of xylose and the accumulation of xylitol was also proven by Tamakawa et al. (2013). The poor ethanol formation and the higher xylitol concentration was explained by a NADPH dependent XR and a NAD^+ dependent XDH, which leads to an imbalance of the coenzymes (Tamakawa et al., 2011). The xylitol

concentration and yield of C245 was higher, while C240 also produced a higher ethanol concentration than C245, but too low for an economical use. This was the reason why C240 was sorted out and the following experiments were done with the yeast strain C245.

Table 59: C240 in the culture bottles with Xall-medium A and B. Maximum produced concentrations of the desired products ethanol and xylitol and the respective yield.

Yeast strain	Run duration [h]	Medium	max. Ethanol [g/L]	max. Ethanol yield [g EtOH /g sugar]	max. Xylitol [g/L]	max. Xylitol yield [g Xylitol /g Xylose]
C240	353	Xall A	**2,85**	0,14	**19,95**	0,72
		Xall B	**3,22**	0,21	**16,18**	0,65

In the experiments in with hydrolysate-medium, the yeast strain **C255/ *Candida lignohabitans*** was sorted out. This yeast strain was further tested in hydrolysate-medium, due to the high ethanol concentration (A 5,8 g/L; B 5 g/L) and a moderate xylitol yield (medium A 0,51 g xylitol/g xylose and medium B 0,55 g/g xylose) in Xall-medium. In hydrolysate-medium the xylose degradation was still fast, but C255 formed 50% more biomass (biomass yield 0,25-0,28 g CDM/g sugar) and around 50% less ethanol and xylitol. For the production of ethanol or xylitol the other yeast strains were more efficient and for this reason C255 was not further tested.

In the literature *Candida lignohabitans* is also known as *Sugiyamaella lignohabitans* (Bellasio et al., 2016). C255 is described to utilize C5 sugars with a high rate, even in hemicellulose hydrolysate (Cassa-Barbosa et al., 2015, Bellasio et al., 2016). In the current studies *C. lignohabitans* was rather used for the production of organic acids from lignocellulosic material (Bellasio et al., 2015). Nevertheless, *C. lignohabitans* Y1757 was examined on the production of ethanol from xylose, but the yeast showed only a low ethanol yield of 0,068 g ethanol/g xylose and a xylitol yield of 0,627 g xylitol/g xylose (Sena et al., 2017). The same author reported a xylose degradation of 89,4%.

The following yeast strains of the pretests were selected for further testing in fermenter runs:

- A027 was selected due to the high ethanol yield in the pretests with Xall-medium
- C238 showed a fast xylose degradation and an average ethanol and xylitol yields
- C245 reached the highest xylitol yields of all yeast strains and showed fast xylose degradation rates

The results are discussed in chapter 6.2 and 6.3.

6.2 Overall observations in the pretests & fermenter runs

Dependent on the medium ingredients, the pH-value was different. In the pretests with Xall-medium, peptone and yeast extract lowered the decrease of the pH-values by minimum 0,13 pH-units (C240) up to 1,19 pH-units (A027). This suggests, that peptone and yeast extract have a buffer capacity, which was also presumed by Thomas et al. (2002). While in the experiments in the culture bottles with Xall-medium the pH-value decreased 0,4-3,1 pH-units, it dropped only 0,4-1,1 pH-units in the hydrolysate-medium. The hydrolysate seems to contain ingredients which have a positive influence on the pH-value.

For none of the experiments a correlation between the drop of the pH-value and the maximum produced acetic acid or other organic acids was detected. It can be assumed, that the pH decreased due to nitrogen assimilation during the growth (Helle and Duff, 2004). The hydrolysate might contain additional nitrogen sources, which could be used of the yeast cells. A low pH can have a negative impact on the xylose degradation or ethanol formation (Helle and Duff, 2004, Stambuk et al., 2008). In the experiments, with Xall-medium, were the pH dropped a lot, a neutralization could be carried out to exclude this problem.

For a cost-effective conversion, the complete fermentation of all sugars in the hydrolysate is essential (Madhavan et al., 2012). For the pre-experiments with both types of media, the co-metabolism of C6 sugars seems to be beneficial for the xylose degradation (except of A027 in the hydrolysate-medium). As described in chapter 3.4.1, there are two different kinds of xylose transport systems. The C6 sugars, especially glucose, might influence the shared low affinity facilitated diffusion transporter in the xylose consumption rate. After the fully degradation of the C6 sugars, the yeast cells probably switch to the high affinity xylose H^+ symport system, which has a different uptake rate (Leandro et al., 2006, Stambuk et al., 2008, Madhavan et al., 2012). In contrast, Madhavan et al. (2012) described for *S. cerevisiae* a slower rate of xylose transport in presence of glucose. This could be another reason, beside of the already poor xylose degradation, why the yeast strain A027 showed no effect of co-metabolism in hydrolysate-medium.

Other than in the pretests, the presence of C6 sugars seems to have no influence on the xylose degradation in the fermenter runs, whether with Xall-medium or with hydrolysate-medium. Instead a faster xylose degradation was found during the higher xylose concentration of around 100 g/L at the beginning of the feed-parts (see chapter 5.2.1.4 and 5.2.2.3). As described in chapter 3.5.2, the initial xylose concentration is an important factor on the activity of the xylose metabolizing enzymes XR and XDH (McMillan, 1993, Winkelhausen and Kuzmanova, 1998). This means that more xylose can be metabolized when the concentration is high. According to Winkelhausen and Kuzmanova (1998), a xylose concentration between 100-200 g/L also increases the formation of xylitol instead of ethanol. McMillan (1993) also describes the phenomena of a lower ethanol yield and higher xylitol yield at xylose concentrations around 100 g/L. The same author also describes, that few of the examined yeast strains (in this case *C. shehatae*, *P. stipitis*, *P. tannophilus*) could fully utilize the xylose, when given at this higher initial concentration.

An overall phenomenon was the xylose degradation rate in the culture bottles of A027, C238 and C245 with hydrolysate-medium, which was the lowest of all experiments. A reason could be the ingredients of the hydrolysate-medium combined with the lower oxygen-input into the culture bottles. The oxygen input also seems to be the reason for the increased xylose degradation rate in the fermenter runs (see chapter 3.4.3).

The xylose degradation rate in the culture bottles was also 3 times lower than in the fermenter runs with the hydrolysate-medium. An explanation could be differences in composition of the wheat straw hydrolysate, which was prepared in two independent batches.

Arabinose was for all yeast strains and in all experiments the least favored sugar. The arabinose degradation in pretests with Xall-medium (see Table 60) also showed an amplified co-metabolism

effect for the yeast strain A027. For the fermentation of hydrolysate-medium in the pretests, the presence of glucose and xylose had a positive effect on the arabinose degradation rate of all tested yeast strains. The stronger arabinose degradation rate can also be explained by the impact of the C6 sugars and the xylose on the arabinose transporters, which are similar arranged like the xylose transporter systems (see chapter 3.4.1).

Table 60: Selected yeast strains in the culture bottles with Xall-medium A and B. Overview of the calculated arabinose degradation rates with C6 sugars and xylose, with xylose and without any other sugar.

Yeast strain	Run duration [h]	Medium	Δ Arabinose with C6+Xylose [g/L/h]	Δ Arabinose with Xylose [g/L/h]	Δ Arabinose without Xylose [g/L/h]
A027	497	Xall A	0,014	0,004	-
		Xall B	0,011	0,004	-
C238	476,5	Xall A	0,003	0,003	0,001
		Xall B	0,005	0,004	0,004
C240	353	Xall A	0,001	0,001	0,005
		Xall B	0,000	0,001	-
C245	353	Xall A	0,001	0,001	0,007
		Xall B	0,003	0,001	0,001
C255	497	Xall A	0,003	0,006	-
		Xall B	0,003	0,005	0,014
HA1129	503,25	Xall A	0,001	0,001	-
		Xall B	0,001	0,001	-

The CDM yield for the pretests in Xall- and hydrolysate-medium (see Table 61 and Table 62) showed a higher CDM yield for the C5 sugars, than for the C6 sugars (see chapter 5.1.1, chapter 5.1.2) It can be assumed that the yeast cells prefer to use the C6 sugars for the formation of ethanol than for biomass. This could be the reason why the yeast strains in the pretests of both media types, except of A027 Xall-medium B, only used the available C6 sugars for the formation of ethanol.

Table 61: Selected yeast strains in the culture bottles with Xall-medium A and B (with peptone and yeast extract). Overview of the calculated CDM yields: at the END with C6+C5 sugars and between 0 g/L C6 and the END only from C5 sugars.

Yeast strain	Run duration [h]	Medium	CDM yield [g CDM /g C6+C5] END	CDM yield [g CDM/g C5] between 0g/L C6 and END
A027	497	Xall A	0,13	0,17
		Xall B	0,11	0,14
C238	476,5	Xall A	0,19	0,29
		Xall B	0,19	0,29
C240	353	Xall A	0,05	0,05
		Xall B	0,07	0,05
C245	353	Xall A	0,04	0,23
		Xall B	0,04	0,10
C255	497	Xall A	0,14	0,24
		Xall B	0,14	0,27
HA1129	503,25	Xall A	0,13	0,17
		Xall B	0,14	0,15

The fermenter runs showed a contradictory outcome, as the CDM yield for all sugars was in the batch-parts higher than for the C5 sugars. Regardless of this observation, none of the tested yeast strains in the fermenter runs produced ethanol from C5 sugars during the presence of C6 sugars.

Table 62: Selected yeast strains in the culture bottles with hydrolysate-medium A and B. Overview of the calculated CDM yields: at the END with C6+C5 sugars and between 0 g/L C6 and the END from C5 sugars.

Yeast strain	Run duration [h]	Medium	CDM yield [g CDM /g sugar] END	CDM yield [g CDM/g C5] between 0g/L C6 and END
A027	473,5	Hydrolysate A	0,16	0,49
		Hydrolysate B	0,15	0,39
C238	473,5	Hydrolysate A	0,25	0,35
		Hydrolysate B	0,26	0,42
C245	473,5	Hydrolysate A	0,12	0,20
		Hydrolysate B	0,28	0,53
C255	475	Hydrolysate A	0,25	0,32
		Hydrolysate B	0,28	0,36

After the fully degradation of the C6 sugars, none of the yeast strains tested in the culture bottles, formed ethanol from the pentose sugars with an efficient yield (see chapter 5.1.1 and 5.1.2; see Table 63 and Table 64).

Table 63: Selected yeast strains in the culture bottles with Xall-medium A and B. Ethanol yield calculated with total sugar consumption (at the time point of maximum ethanol concentration), with C6+C5 and C6 consumption (both at 0 g/L C6 sugars) and the ethanol yield of C5 sugars between 0 g/L and the maximum ethanol concentration.

Yeast strain	Run duration [h]	Medium	Ethanol yield [g EtOH /g sugar] MAX	Ethanol yield [g EtOH /g C6+C5] at C6 0 g/L	Ethanol yield [g EtOH /g C6] at C6 0 g/L	Ethanol yield [g EtOH /g C5] between 0g/L C6 and MAX
A027	497	Xall A	0,21	0,23	0,44	0,15
		Xall B	0,22	0,25	0,58	0,16
C238	476,5	Xall A	0,10	0,19	0,36	0,03
		Xall B	0,14	0,22	0,39	0,04
C240	353	Xall A	0,14	0,17	0,29	0,03
		Xall B	0,21	0,21	0,35	-
C245	353	Xall A	0,06	0,06	0,22	-
		Xall B	0,07	0,07	0,24	0,01
C255	497	Xall A	0,15	0,16	0,37	0,13
		Xall B	0,12	0,14	0,36	0,07
HA1129	503,25	Xall A	0,22	0,25	0,36	0,01
		Xall B	0,28	0,28	0,40	-

Table 64: Selected yeast strains in the culture bottles with hydrolysate-medium A and B. Ethanol yield calculated with total sugar consumption (at the time point of maximum ethanol concentration), with C6+C5 consumption and C6 consumption (both at 0 g/L C6 sugars).

Yeast strain	Medium	Ethanol yield [g EtOH /g sugar] MAX	Ethanol yield [g EtOH /g C6+C5] at C6 0 g/L	Ethanol yield [g EtOH /g C6] at C6 0 g/L	Ethanol yield [g EtOH /g C5] between 0g/L C6 and MAX
A027	Hydrolysate A	0,27	0,25	0,27	0,44
	Hydrolysate B	0,26	0,24	0,26	0,46
C238	Hydrolysate A	0,16	0,25	0,28	0,10
	Hydrolysate B	0,21	0,24	0,27	0,17
C245	Hydrolysate A	0,13	0,13	0,21	-
	Hydrolysate B	0,14	0,14	0,24	0,03
C255	Hydrolysate A	0,08	0,15	0,23	0,05
	Hydrolysate B	0,08	0,15	0,23	0,04

6.3 Discussion of the performance of the three main yeast strains

6.3.1 A027/ *Saccharomyces cerevisiae* BP10001

The metabolic engineered yeast strain A027 (see chapter 4.1) was tested on ethanol production. It showed the highest ethanol production of all tested yeast strains in the pretests in Xall-culture bottles (see Table 65). Peptone and yeast extract seem to have a positive effect on the ethanol formation of A027 (Xall-medium A: 6,63 g ethanol/L, Xall-medium B: 8,08 g ethanol/L), while the ethanol yield was around 0,21-0,22 g/g for Xall-medium A and B. The ethanol was formed from C6 and C5 sugars. The yield from C5 sugars after the degradation of the C6 sugars was around 0,16 g ethanol/g C5, which shows that A027 can also degrade pentose sugars.

In the fermenter runs with Xall-medium B, A027 also showed the highest ethanol concentration of all yeast strains in this experiment (10,11 g/L batch-part; 3,02 g/L feed-part). The maximum ethanol yields of all sugars in the fermenter runs in the Xall-medium were a little bit higher than in the culture bottles, but the yield of the C5 sugars (after the degradation of the C6 sugars) was significantly higher. A027 was the only yeast strain in the fermenter runs with Xall-medium, which produced ethanol with a noticeable yield of 0,25 g ethanol/g C5 sugars after the fully degradation of C6 sugars. The conditions of the fermenter, like the controlled oxygen supply seem to have a positive impact on the ethanol formation from xylose.

Table 65: Conclusion of the ethanol and xylitol concentrations and yield of the pretests and fermenter runs with Xall-medium and hydrolysate-medium for the yeast strain A027.

Experiment	Medium		Δ max. Ethanol [g/L]	max. Ethanol yield [g/g]	Ethanol yield [g EtOH /g C5] between 0g/L C6 and MAX	Δ max. Xylitol [g/L]	max. Xylitol yield [g/g]
Culture bottles	Xall A		6,63	0,21	0,15	4,96	0,23
	Xall B		8,08	0,22	0,16	5,84	0,24
Fermenter	Xall B	Batch	10,11	0,29	0,25	2,28	0,11
		Feed	3,02	0,25	0,25	0,12	0,02
Culture bottles	Hydrolysate A		3,98	0,27	0,44	0,16	0,06
	Hydrolysate B		4,28	0,26	0,46	0,19	0,04

Compared to the other yeast strains the xylitol concentration of A027 in the culture bottles with Xall-medium was low (4,96 g/L in Xall A, 5,84 g/L in Xall B). This results match with the outcome of Novy et al. (2014), who described a more than halved xylitol yield of the metabolic engineered *S. cerevisiae* A027 compared to the wild type BP000. In the fermenter with Xall-medium B, the xylitol concentration and yield was even lower with 2,28 g/L in the batch and 0,12 g/L in the feed-part. The xylitol yield of the batch-part was with 0,11 g/g the half of the yield in culture bottles. In the feed-part the xylitol yield was 0,02 g/g. This could be related to the different oxygen-input into the fermenter, due to the strong influence of oxygen on the ethanol and xylitol formation (see chapter 3.4.3).

Having a look on Table 66, the biomass yields of A027 with Xall-medium in the culture bottles were in the middle range (0,11-0,13 g CDM/ g sugar), but were lower in the fermenter experiments (0,02 g CDM/ g sugar in the batch and 0,09 g CDM/ g sugar in the feed-part).

Table 66: Conclusion of the biomass yield, the xylose degradation rate and the percentage of degraded xylose of the pretests and fermenter runs with Xall-medium and hydrolysate-medium for the yeast strain A027.

Experiment	Medium		Biomass yield [g CDM/g sugar]	Δ Xylose [g/L/h]	Δ Xylose 305h [%]	Δ Xylose End [%]
Culture bottles	Xall A		0,13	0,06	66,70	98,40
	Xall B		0,11	0,06	75,60	100,00
Fermenter	Xall B	Batch	0,09	0,08	-	87,90
		Feed	0,02	0,06	-	13,15
Culture bottles	Hydrolysate A		0,16	0,01	11,50	17,60
	Hydrolysate B		0,15	0,01	13,10	20,00

One disadvantage is the poor xylose degradation of A027 (see Table 66). The experiments for A027 with Xall-medium showed in the culture bottles and in the fermenter runs the lowest xylose degradation rate of all yeast strains (0,06 g/L/h for the culture bottles and the feed-part of the fermenter runs, 0,08 g/L/h in the batch-part of the fermenter runs). Also, the arabinose degradation was the slowest of all yeast strains.

The higher ethanol concentration, low xylitol concentration and poor xylose degradation coincides with the results of Novy et al. (2014), who reported a two magnitude lower xylose uptake rate than the glucose uptake rate. Novy et al. (2014) described the low xylose degradation rate as a mutual problem of genetic modified *S. cerevisiae.* Which is according to this author independent of strategy used for the metabolic engineering.

The metabolic engineering could also have an impact on the fermentation with hydrolysate-medium. With a rate of 0,01 g/L/h the pretests with hydrolysate-medium showed an even worse xylose degradation than the experiments with Xall-medium (see Table 66). A maximum of only 20% xylose was degraded in 473,5 hours. This can also be seen in the lowest xylitol yield of all conducted experiments, which was around 0,04-0,06 g xylitol/g xylose (see Table 65).

The formed ethanol concentration with hydrolysate-medium in the culture bottles was around 3,98 g/L in hydrolysate-medium A and 4,28 g/L in hydrolysate-medium B (see Table 65). This ethanol concentration was the second highest after C238, of the yeast strains tested with hydrolysate-medium. The ethanol yield of all sugars was between 0,26-0,27 g ethanol/g sugar and therefore similar to the one in the fermenter runs with Xall-medium. However, the ethanol yield from the C5 sugars after the fully degradation of the C6 sugars was with 0,44-0,46 g ethanol/g C5 sugars the highest of all yeast strains and all conducted experiments. Due to the low xylose degradation rate, the most ethanol was produced from glucose and the formed ethanol concentration is still too low for an economical usage. For this reason, the yeast strain A027 becomes irrelevant for the following experiments with hydrolysate-medium.

The reason for the worsened xylose fermentation behavior in hydrolysate-medium compared to the Xall-medium, could be the osmotic and ionic strength of the hydrolysate. Because of the neutralization of the hydrolysate with sodium hydroxide (NaOH), the medium contains a high concentration of sodium chloride (NaCl). This creates a high ionic strength or high osmotic pressure in the medium. It seems the genetically modified *S. cerevisiae* strain A027 is not able to grow on xylose under this condition. The inhibition of the fermentation of S. cerevisiae or other ethanologens by

osmotic stress was also described by Helle and Duff (2004). These authors explained the consequence of osmotic stress by the establishment of an osmotic pressure gradient across the cell membrane. Impermeable solutes or solutes which cannot be transported by the cell through the cell membrane, the balance of the osmotic pressure is regulated by the release of water of the cell and the shrinking of the cell volume. This effect leads to a toxic effect and an increase in energy demand of the cell. For solutes which can cross the cell membrane, the inhibition according to Helle and Duff (2004) could be an increase in maintenance energy demand, due to the required pump out of the cell into the medium against a concentration gradient. What speaks against this theory of osmotic stress influence on A027, is the fact that the increasing ATP demand was stated by Maiorella et al. (1984) to lead to less biomass formation, but a higher ethanol production. However, the biomass yield of A027 in the culture bottles with hydrolysate-medium was the highest of all experiments carried out with this yeast strain (see Table 66).

Another explanation for the inhibition could be the higher overall sensitivity to salts than to sugars, correlating with the water activity, also described by Maiorella et al. (1984). Due to the binding of the cations to the proteins, the enzyme activity can be disturbed.

To improve the fermentation of hydrolysate-medium with A027, more tests should be done (see suggestions in chapter 6.4.

6.3.2 C238/ unknown strain

The unknown yeast strain C238 showed one of the two fastest xylose degradation rates of all strains tested in the Xall-medium in the culture bottles (see Table 67). Whereas the rate in the culture bottles with hydrolysate-medium was 50% lower than with Xall-medium (reason described in chapter 6.2). The runs with Xall-medium showed with 0,17 g/L/h in the batch-part and 0,27 g/L/h the fastest xylose degradation of all experiments carried out for C238. The fermenter runs with hydrolysate-medium showed rates of 0,15 g/L/h in the batch and 0,19 g/L/h in the feed-part. The higher xylose degradation rate in the feed-part is noticeable and seems to be related to the higher CDM in the feed-part (see Table 68 and Table 69) and the higher xylose concentration (described in chapter 6.2).

Table 67: Conclusion of the biomass yield, the xylose degradation rate and the percentage of degraded xylose of the pretests and fermenter runs with Xall-medium and hydrolysate-medium for the yeast strain C238.

Experiment	Medium		Biomass yield [g CDM/g sugar]	Δ Xylose [g/L/h]	Δ Xylose 305h [%]	Δ Xylose End [%]
Culture bottles	Xall A		0,19	0,09	98,90	100,00
	Xall B		0,19	0,09	100,00	100,00
Fermenter	Xall B	Batch	0,07	0,17	-	98,20
		Feed	0,06	0,27	-	89,05
Culture bottles	Hydrolysate A		0,25	0,05	54,80	83,80
	Hydrolysate B		0,26	0,04	42,30	66,20
Fermenter	Hydrolysate B	Batch	0,10	0,15	-	88,45
		Feed	0,04	0,19	-	39,90

Table 68: Xylose degradation rate per CDM [g/h/g CDM], specific in [g/g CDM] and [g/h] in the first run of C238 in the fed-batch process in the fermenter with Xall-medium B.

Process	Ø CDM [g/L]	Δ Duration [h]	Δ Xylose [g/L]	Δ Xylose [g/g CDM]	Δ Xylose [g/g CDM/h]	Δ Xylose [g/L/h]
Batch	1,66	137,00	20,09	12,105	0,088	0,147
	2,53	56,25	8,91	3,516	0,063	0,158
Feed	4,34	87,25	58,26	13,424	0,154	0,668
	6,21	24,00	1,74	0,280	0,012	0,073
	6,38	96,25	30,16	4,725	0,049	0,313
	8,40	71,75	2,26	0,269	0,004	0,032
			Mean value	**5,720**	**0,062**	**0,232**

Table 69: Xylose degradation rate per CDM [g/h/g CDM], specific in [g/g CDM] and [g/h] in the second run of C238 in the fed-batch process in the fermenter with Xall-medium B.

Process	Ø CDM [g/L]	Δ Duration [h]	Δ Xylose [g/L]	Δ Xylose [g/g CDM]	Δ Xylose [g/g CDM/h]	Δ Xylose [g/L/h]
Batch	2,07	148,00	27,64	13,346	0,090	0,187
Feed	3,19	14,75	2,05	0,642	0,044	0,139
	4,72	120,00	37,54	7,950	0,066	0,313
	5,51	192,00	29,18	5,295	0,028	0,152
			Mean value	**6,808**	**0,057**	**0,198**

The effect of oxygen input on the xylose degradation in the fermenter runs with Xall-medium, was identified during clogging of the air-sparger. The lower air-input lead to a decreased xylose degradation rate. The need of oxygen input for a good xylose degradation and metabolism is described in chapter 3.4.3. For xylose-degrading yeasts an oxygen limitation is recommended to maintain a good fermentation and simultaneous growth (Kuriyama and Kobayashi, 1993).

Compared to the other tested yeast strains, C238 showed the highest biomass yields in the culture bottle experiments (see Table 67). The highest biomass yields of 0,25 g/g without and 0,26 g/g with peptone and yeast extract, was reached in the hydrolysate-medium pretests. The yeast strains A027, C238 and C245 also showed the highest biomass yield in the pretests with hydrolysate-medium. Looking at Table 67, Table 66 in chapter 6.3.1 and Table 72 in chapter 6.3.3, it seems the hydrolysate-medium in the pretest decreased the xylose degradation rates of the yeast strains, while the biomass yields were increased. In contrast to this observation, the biomass yield of C238 in the fermenter runs was more than 50% lower than the appendant pretests (Xall-medium B: 0,07 g/g batch, 0,06 g/g feed; hydrolysate-medium B: 0,10 g/g batch, 0,04 g/g feed).

As described for the pretests in chapter 6.2, the ingredients of the hydrolysate-medium seems to have a positive influence on the pH-value. The same observation was found for C238 in the fermenter runs. While in Xall-medium the pH decrease was between 0,6-2,4 units, it only decreased 0,3-0,5 units in the hydrolysate-medium. This effect is positive, because no further neutralization of the culture broth is needed to prevent negative effects of the decreasing pH-value.

The pretests showed, that C238 can produce ethanol, but also xylitol with an average yield. In the Xall-medium pretests the maximum ethanol concentration was 4,11 g/L in Xall-medium A and 5,56 g/L in Xall-medium B. The ethanol concentration in the hydrolysate-medium pretests was a little bit higher with a concentration of 5,59 g/L in hydrolysate-medium A and 5,93 g/L in hydrolysate-medium B. The

reached ethanol concentration was the highest of all yeast strains in the pretests with hydrolysate-medium.

The ethanol yield from all sugars was higher in the pretests with hydrolysate-medium, while for both media the addition of peptone and yeast extract increased the ethanol yield (see Table 70). Most of the ethanol was formed from C6 sugars, but after the fully degradation of the hexoses the ethanol was also formed from the C5 sugars with a lower yield. The ethanol yield from C5 sugars in the pretests with hydrolysate-medium B was the highest of all experiments with C238.

Table 70: Conclusion of the ethanol and xylitol concentrations and yield of the pretests and fermenter runs with Xall-medium and hydrolysate-medium for the yeast strain C238.

Experiment	Medium		Δ max. Ethanol [g/L]	max. Ethanol yield [g/g]	Ethanol yield [g EtOH /g C5] between 0g/L C6 and MAX	Δ max. Xylitol [g/L]	max. Xylitol yield [g/g]
Culture bottles	Xall A		4,11	0,10	0,03	10,96	0,40
	Xall B		5,56	0,14	0,04	10,49	0,37
Fermenter	Xall B	Batch	4,19	0,11	0,03	15,73	0,56
		Feed	9,15	0,11	0,11	37,92	0,49
Culture bottles	Hydrolysate A		5,59	0,16	0,10	1,95	0,08
	Hydrolysate B		5,93	0,21	0,17	1,82	0,09
Fermenter	Hydrolysate B	Batch	3,82	0,20	0,03	13,56	0,53
		Feed	14,36	0,22	0,09	22,52	0,64

In the fermenter runs with Xall-medium B, the mean ethanol concentration was 4,19 g/l in the batch-parts and additional 9,15 g/L in the feed-parts. The fermenter runs with hydrolysate-medium B reached an ethanol concentration of 3,82 g/L in the batch-part and additional 14,36 g/L in the feed-part. It must be emphasized that no additional glucose was added in the feed of the Xall-medium runs, while in the hydrolysate-medium runs an amount of glucose according to the hydrolysate composition was added. This means, that the formed ethanol in the feed-part of the Xall-medium runs was fully formed from the C5 sugars. The higher ethanol concentration in the feed-part of the fermenter runs with hydrolysate-medium, seem to be a result of the higher amount of glucose added to the fermentation.

While the ethanol yield of the Xall-medium fermenter runs was with 0,11 g ethanol/g sugar in the same range as in the appendant pretests, the runs with hydrolysate-medium had a 50% higher ethanol yield (0,20 g ethanol/g sugar in the batch; 0,22 ethanol/g sugar in the feed-part). In presence of C6 sugars, no ethanol was formed from xylose, the biggest part of ethanol was formed from the C6 sugars. Nevertheless, also C5 sugars were used for the formation of ethanol, but with a lower yield. In contrast to the different yield from all sugars, the yield from the C5 sugars was for the Xall-and Hydrolysate-medium similar with 0,03 g ethanol/g C5 sugars in the batch-parts and around 0,10 g ethanol/g C5 sugars in the feed-parts. The ethanol yield from C5 sugars was not affected by the additional glucose in the feed of the hydrolysate-medium runs.

While the addition of peptone and yeast extract to the medium increased the ethanol concentration and yield in the pretests, it also seems to slightly decrease the xylitol concentration. The xylitol concentration in the pretests was for Xall-medium A 10,96 g/L and Xall B 10,49 g/L. For the

fermenter runs with Xall-medium B the mean xylitol concentrations of 15,73 g/L in the batch-part were measured and 37,92 g/L were additionally formed in the feed-part. The xylitol yield in the fermenter runs of Xall-medium was with 0,56 g xylitol/g xylose in Xall-medium A and 0,49 g xylitol/g xylose in Xall-medium B around 0,15 g/g (32-40%) higher than in the culture bottles. This could be a result of the controlled aeration rate in the fermenter runs, due to the strong influence of oxygen on the xylitol formation (see chapter 3.4.3).

In the pretests with hydrolysate-medium a remarkable difference of the xylitol concentration was measured (see Table 70). For the hydrolysate-medium A 1,95 g/L was measured, while for the medium with peptone and yeast extract the xylitol concentration was 1,82 g/L. The xylitol yields also significantly decreased from 0,40 g/g in Xall-medium A to 0,08 g/g in hydrolysate-medium A and 0,37 g/g in Xall-medium B to 0,09 g/g in hydrolysate-medium B. As described by Olofsson et al. (2008) the strong decrease of xylitol formation could be a consequence of additional electron acceptors present in the hydrolysate-medium. The electron acceptors change the redox imbalance and help to regenerate the NAD^+, without the regeneration this usually leads to the excretion of the intermediate xylitol (Madhavan et al., 2012) (for more information to the sugar metabolism see chapter 3.4.2).

Looking at the fermenter runs with hydrolysate-medium, the xylitol concentration of the batch-part was 13,56 g/L and additionally 22,52 g/L were formed in the feed-part. This significant difference of the xylitol formation can also be seen in the xylitol yield. In the batch-part of the fermenter runs with hydrolysate-medium C238 reached 0,53 g xylitol/g xylose, while in the feed-part it was 0,64 g xylitol/g xylose. The xylitol concentration was in this case twice as high as the formed ethanol concentration. The much higher xylitol yield compared to the pretests could probably be a result of the different oxygen input, the consequently higher xylose degradation rate and/or maybe a different composition of the hydrolysate-medium with less electron acceptors.

Compared to the xylitol yield, the ethanol yield of the Xall-medium experiments and the fermentation runs with hydrolysate-medium, was too low and inefficient for an economical usage.

Beside of the inefficient ethanol yields, the by-product formation has risen more in the fermenter runs than in the pretests. In the fermenter runs with Xall-medium, the highest by-product concentration was of arabitol/mannitol, which reached a concentration of 4,7 g/L in the feed-part of the first run and 2,7 g/L in the second run. In the fermenter runs with hydrolysate-medium the highest side product was glycerol, with a maximum concentration of 4,1 g/L. The second highest was arabitol/mannitol with a maximum concentration of 2,3 g/L. While there was no explanation found for the higher formation of arabitol/mannitol, the glycerol formation in the hydrolysate-medium runs could be another way for the yeast cells to balance the redox imbalance and to regenerate NAD^+. This effect was reported by Jeffries (2006) for *S. cerevisiae* under oxygen-limited conditions. According to the author, *S. cerevisiae* usually tries to compensate a redox imbalance with the formation of glycerol, but during the assimilation of xylose it is not sufficient and the yeasts instead form xylitol. This could also explain the stronger rise of the glycerol concentration directly after the addition of the sugar feed, when the glucose concentration was the highest in the fermenter runs in the hydrolysate-medium. After the fully degradation of the glucose the glycerol concentration remained relatively constant.

6.3.3 C245/ *Candida* sp.

Candida sp.C245 was identified to be the favored yeast strain for xylitol formation, due to the highest xylitol concentration and yields in the pretests (see Table 71).

Table 71: Conclusion of the concentrations of ethanol, xylitol and glycerol and the yield of ethanol and xylitol in the pretests and fermenter runs with Xall-medium and hydrolysate-medium for the yeast strain C245.

Experiment	Medium		Δ max. Ethanol [g/L]	max. Ethanol yield [g/g]	Ethanol yield [g EtOH /g C5] between 0g/L C6 and MAX	Δ max. Xylitol [g/L]	max. Xylitol yield [g/g]	max. Glycerol [g/L]
Culture bottles	Xall A		2,11	0,06	-	22,28	0,81	3,45
	Xall B		2,26	0,07	0,01	22,54	0,83	2,51
Fermenter	Xall B	Batch	1,44	0,06	-	20,86	0,73	5,72
		Feed	0,01	-	-	53,21	0,69	19,46
Culture bottles	Hydrolysate A		2,79	0,13	-	13,39	0,64	4,32
	Hydrolysate B		3,40	0,14	0,03	6,35	0,31	2,14
Fermenter	Hydrolysate A	Batch	2,22	0,08	0,01	10,59	0,46	2,95
		Feed	13,49	0,11	0,04	39,49	0,46	5,88

The pretests with Xall-medium showed with 0,81 g xylitol/g xylose with Xall-medium A and 0,83 g xylitol/g xylose with Xall-medium B the highest maximum xylitol yield of all experiments with C245, near the theoretical maximum xylitol yield. With this yield a xylitol concentration of 22,28 g/L with Xall-medium A and 22,54 g/L with Xall-medium B was reached. It seemed the presence of peptone and yeast extract in Xall-medium B had a slightly positive effect on the formation of xylitol.

The fermenter runs with Xall-medium B only showed in the batch-part a maximum xylitol concentration of 20,86 g/L and in the feed-part additionally 53,21 g/L xylitol were formed. Compared to the culture bottles with Xall-medium B, the xylitol yield was considerable lower for the fermenter runs (batch-part: 0,73 g xylitol/g xylose; feed-part: 0,69 g xylitol/g xylose).

The experiments of C245 with Xall-medium showed the highest xylose degradation rate of all yeast strains in the culture bottles and in the fermenter runs (see Table 72). In the tests with hydrolysate-medium the xylose degradation rate in the fermenter runs was high in the batch-parts and with 0,34 g/L/h the highest calculated in the feed-parts. The high xylose degradation rate in the feed-part of the fermenter was reached by the higher total biomass concentration in the fermenter, which was formed over the long fermentation time. In contrast the xylose degradation was much lower in the pretests with hydrolysate-medium.

The lower xylose degradation also influenced the xylitol concentration, which was 13,39 g/L in the medium without and 6,35 g/L with peptone and yeast extract. Unlike the observation in the Xall-medium pretests, peptone and yeast extract had a strong negative impact on the xylitol yield in the hydrolysate-medium, which was 50% lower than without (without: 0,64 g xylitol/g xylose; with peptone and yeast extract: 0,31 g xylitol/g xylose). At the same time the biomass yields were 50% higher (see Table 72) and therefore the highest measured biomass yields in the experiments with C245. Compared to the other experiments of C245, the calculated biomass yields for the pretests in hydrolysate-medium A were already three times higher. Beside of C245, the same negative effect of

peptone and yeast extract was also found in the pretests in hydrolysate-medium of yeast strain C255 (see chapter 5.1.2).

Table 72: Conclusion of the biomass yield, the xylose degradation rate and the percentage of degraded xylose of the pretests and fermenter runs with Xall-medium and hydrolysate-medium for the yeast strain C245.

Experiment	Medium		Biomass yield [g CDM/g sugar]	Δ Xylose [g/L/h]	Δ Xylose 305h [%]	Δ Xylose End [%]
Culture bottles	Xall A		0,04	0,14	95,80	98,30
	Xall B		0,04	0,08	96,80	99,00
Fermenter	Xall B	Batch	0,04	0,25	-	97,80
		Feed	0,01	0,24	-	90,05
Culture bottles	Hydrolysate A		0,12	0,05	68,20	78,10
	Hydrolysate B		0,28	0,05	63,40	79,80
Fermenter	Hydrolysate A	Batch	0,05	0,18	-	95,05
		Feed	0,04	0,34	-	93,05

Due to the negative impact of the additives, hydrolysate-medium A was used for the fermenter runs of C245. The xylitol yield was despite this choice lower than in the pretests and was for the batch- and feed-part around 0,46 g xylitol/g xylose. The reached xylitol concentration was therefore 10,59 g/L in the batch-part and additionally 39,49 g/L in the feed-part, which was lower than in the fermenter runs with Xall-medium B. As already described for C238 in chapter 6.3.2., the lower xylitol concentration in the hydrolysate-medium can be explained by the presence of electron acceptors, which change the redox imbalance (Olofsson et al., 2008, Madhavan et al., 2012).

Another reason for the much lower xylitol concentration could be the higher ethanol concentration in the feed-part of the fermenter runs with hydrolysate-medium A. Except of the feed-parts of the fermenter runs with hydrolysate-medium A, the ethanol concentration and yield of C245 was for all experiments the lowest of all tested yeast strains (see Table 71). The feed-part in the fermenter runs with hydrolysate-medium reached an ethanol concentration of 13,49 g/L, which is similar to the ethanol concentration formed from C238 in the same experiment. The maximum ethanol yield of C245 of 0,14 g ethanol/g sugar, was reached in the pretests with hydrolysate-medium B. The ethanol in all experiments with C245 was nearly only formed from the C6 sugars, hence the ethanol yield with C5 sugars was the lowest of all tested yeast strains (maximum 0,04 g ethanol/g C5 sugars). In the feed-part of the fermenter runs with Xall-medium B, nearly no ethanol was formed, since there was no glucose included in the added sugar feed. Instead, C245 started to degrade the accumulated ethanol in the feed-part of the fermenter runs.

Having a look on the by-product formation of C245 in the different experiments compared to the other yeast strains, the very high concentration of glycerol was detected (see Table 71). In the pretests the concentration was similar in the Xall-medium and hydrolysate-medium, while the glycerol concentration was in the range of 3,5-4,4 g/L in the media without and 2-2,5 g/L in the media with peptone and yeast extract. In the fermenter runs with Xall-medium B, the glycerol concentration linearly increased over the fermentation time (batch: 5,72 g/L; feed-part: 19,46 g/L). It seems to be dependent on the formed xylitol concentration. The situation was different in the fermenter runs with

hydrolysate-medium B. Here the glycerol concentration had the strongest increase at the beginning of the batch-part and after the addition of the sugar feed. Afterwards the concentration was nearly constant over the remaining period. Furthermore, the glycerol concentration was much lower than in the Xall-medium fermenter runs (batch-part: 2,95 g/L; feed-part: 5,88 g/L). It can be assumed, that the glycerol concentration in this experiment was dependent on the added amount of glucose or the ethanol which was formed from the glucose feed.

The role of glycerol formation as byproduct, was described as important response of yeasts on cellular stress situations, like osmotic stress (Flores et al., 2000, Priscila and Felipe, 2008). Also it has been reported, that glycerol is formed to maintain the cytosolic cell redox balance by regenerating NAD^+ in some yeast, like *S. cerevisiae* (Neivogt and Stahl, 1997, Jeffries, 2006). This might also reduce the available NADH, which is important for the xylose reductase (XR) responsible for the conversion step of xylose to xylitol (see chapter 3.4.2). In *Candida guilliermondii* the NAD^+ regeneration leads to a lower xylitol formation, while up to 12% of the xylose is used for the formation of glycerol (Rodrigues et al., 2003, Priscila and Felipe, 2008). Another author described, that a high glycerol concentration could have a positive effect on the xylose uptake of *Candia tropicalis*, which therefore improves the xylitol production rate (Ko et al., 2006).

The dependence of glycerol formation on the glucose or ethanol concentration in the fermenter runs with hydrolysate-medium, could be explained by the increase of osmotic stress during the formation of ethanol. Also the mechanism of NAD^+ regeneration, during the fermentation of glucose, by increasing the glycerol formation instead of the ethanol formation could be a possible explanation. This mechanism was reported for *S. cerevisiae* strains lacking alcohol dehydrogenase (Drewke et al., 1990, Flores et al., 2000).

For the fermenter runs with hydrolysate-medium A, the total measured concentration of the side products arabitol/mannitol were nearly as high as the measured glycerol concentration. The mean maximum formed arabitol/mannitol concentration in the batch-parts was 0,82 g/L and in the feed-parts 7,31 g/L. In the fermenter runs with Xall-medium the maximum reached arabitol/mannitol concentration of the first run was 6,5 g/L, while the second run only had 3,2 g/L. It is not sure why the formation of arabitol/ mannitol strongly increased at the end of the feed-part of the fermenter runs. However, Koganti et al. (2011) outlined the possibility of producing arabitol from glycerol and also xylitol from arabitol. Due to the complicated interactions of cofactors, oxygen availability and redox imbalance, it is possible that the yeast cells of C245 assimilate arabitol as an intermediate.

One way to decrease the arabitol/mannitol concentration in the fermenter runs with Xall-medium A, was the increase of the initial optical density (see chapter 5.2.3.2). With a 3-fold inoculum the ethanol concentration was little bit higher, but the arabitol/mannitol concentration was lowered by 50%. The higher inoculum also strongly increased the xylose degradation rate (batch: 0,34 g/L/h; feed-part: 0,31 g/L/h) and the xylitol yield (batch-part: 0,82 g xylitol/g xylose). However, the higher xylose concentration in the feed-part of the fermentation run seemed to lower the xylitol yield. The lower xylitol yield also could be a consequence of the lowered maximum optical density, which was caused by the higher initial optical density.

6.4 Outlook and recommendations

For an efficient utilization of the wheat straw hydrolysate and the production of ethanol and xylitol, by the examined yeast strains, following research is needed to develop efficient fermentation processes.

None of the executed tests exposed a yeast strain for the efficient ethanol production from wheat straw hydrolysate. The most promising yeast strain to produce ethanol from Xall-medium was A027, but in hydrolysate-medium this strain was unable to ferment C5 sugars. To improve the fermentation, it should be investigated what kept the engineered *S. cerevisiae* strain from producing ethanol from C5 sugars in the hydrolysate. Further tests should be done with lower ionic strength, different sodium chloride concentrations and different ways of neutralization of the hydrolysate-medium. Another solution could be the usage of another preparation process of the wheat straw hydrolysate. For example, with enzymes (see chapter 3.1.2 and 3.1.3) where no neutralization is needed.

One of the most important parameters in the overall fermentation of xylose and arabinose is the oxygen supply, which has an impact on the xylose degradation and the formation of products. Further experiments could reveal the mechanism of oxygen on the xylose degradation, which was also dependent on other parameters, like the metabolism of the yeast strains or the medium.

A limited oxygen supply must be maintained for a high xylitol concentration, but is also recommended for an efficient ethanol production (see chapter 3.4.3). This means the respective optimum oxygen input for the preferred product must be researched for every yeast strain. With the recent conditions C245 seems to be the best xylitol producer in Xall-medium, while C238 was capable of producing ethanol and xylitol, dependent on the used medium. The researches on the oxygen supply could clarify the application of C238. A sequencing of C238, the currently unknown yeast strain, would be useful to draw conclusions and for a better comparison with other references.

The xylose degradation rate of the yeasts also seems to be influenced by the initial xylose concentration in the fermentation media (see chapter 6.2). For a better understanding, further examinations with different xylose concentration should be done.

On the metabolic level, the metabolic flux analysis of the three main yeast strains would be helpful, to find out how the hexose and pentose sugars were converted into the different products. This could be performed by using isotopically labeled (e.g. 13C) sugars. This way, it could be determined how much ethanol is produced from the pentoses and the origin of the by-products glycerol, acetate and arabitol/mannitol could be determined. Further research on the dependence of glycerol formation on the oxygen supply, the added sugars and the redox imbalance (see chapter 6.3.2), could help to lower the by-product formation of the yeast strains C238 and C245.

The effect of peptone and yeast extract on C245, discovered in the hydrolysate-medium experiments, significantly changed the xylitol yield of the experiment. The shift from xylitol formation to biomass production triggered by these additives, could also be a task of further studies. It could be determined based on metabolic flux analysis or by simple fermentation experiments.

7 Conclusion

In this master thesis, the facts of saving resources, the efficient use of raw materials and the bioconversion of lignocellulosic waste materials were considered. Lignocellulosic biomass is abundant (e.g. 430 Mt of wheat straw annually (Talebnia et al., 2010)) and does not compete with human nutrition. The aim was, to examine the pre-screened yeast strains (A027, C238, C240, C245, C255, HA1129) of the Vogelbusch Biocommodities GmbH cell bank, on their ability to ferment wheat straw hemicellulose hydrolysate and produce fuel ethanol or the sweetener xylitol. The suitable yeast for the fermentation of hemicellulose hydrolysate should fulfill several requirements. The products should be formed with a high yield, a high rate and with a low by-product and biomass formation. The pentose and hexose sugars, contained in the hemicellulose hydrolysate, should be fully utilize by the yeast strains. The yeast also should be robust towards the hydrolysate conditions, like the possible contained inhibitors.

For this investigation, the yeast strains were tested on two different media. The “Xall-medium” contained the synthetic hexose sugars (glucose, galactose, mannose) and pentose sugars (xylose and arabinose), while the “hydrolysate-medium” contained the sugars of the wheat straw hemicellulose hydrolysate as carbon source. To find out if the fermentation can be enhanced by the addition of 1 g/L peptone and 0,5 g/L yeast extract, both media were tested on the yeasts in pretests with and without these additives. For the pretests, all yeast strains were tested in small culture bottles with both media. The best yeast strains A027 (*Saccharomyces cerevisiae* BP10001), C238 (unknown strain) and C245 (*Candida* sp.) have been cultivated in fed-batch fermenter runs with synthetic and hydrolysate-medium and a controlled air supply.

In both parts of the experiments the cells were cultivated with a temperature of 32°C, an initial pH-value around 6 and under limited oxygen conditions. The main reason for the importance of the oxygen regulation is the shift from ethanol production to xylitol accumulation. The shift is attributed to the redox imbalance of the cofactors of the xylose reduction by D-xylose reductase (XR) and xylitol oxidation by Xylitol dehydrogenase (XDH) (Novy et al., 2014). The conversion of xylitol into xylulose by XDH is the rate-limiting step of the ethanol production and also the key for a high yield in xylitol accumulation (Parajó et al., 1998a). The yeasts tend to produce ethanol or xylitol from the sugars dependent on the cofactor demand of the XR, the activity of the XDH and the individual oxygen amount for the oxygen-limitation. For more information about the pentose sugar metabolism of yeasts and the importance of oxygen, see chapter 3.4 and Figure 1 on page 21.

The cultivations were sampled up to once a day and were determined on the optical density, pH, cell dry mass and microbial contamination. The substrate and product concentrations were measured by high performance liquid chromatography (HPLC). The hemicellulose hydrolysate was also examined by HPLC, on the possible inhibitor-concentrations formed due to the pretreatment of the wheat straw. No significant inhibitor-concentrations (of furfural, 5-hydroxymethyl furfural, acetate and formic acid) were found in the hydrolysate or the final fermentation medium (see chapter 4.4.6 and in the appendix 9.4). This means, that an inhibitor effect on the fermentations with hydrolysate-medium can be excluded.

For a cost-effective conversion, the complete fermentation of all sugars in the hydrolysate is essential (Madhavan et al., 2012). All tested yeast strains degraded the C6 sugars quickly in 20-250 hours, while the xylose and arabinose degradation was slower, differed from strain to strain and depended on the media. The arabinose was the least favored sugar. The fastest xylose degradation rates with Xall-medium were found for the fermenter runs of C238 (0,17-0,27 g/L/h) and C245 (0,24-0,25 g/L/h). The fastest xylose degradation rates (see Table 73) with hydrolysate-medium were found for the fermenter runs with C245 (0,18-0,34 g/L/h). The xylose degradation in the pretests with hydrolysate-medium was for all yeast strains low (0,01-0,05 g/L/h). This could be explained by the lower oxygen-input in the pretests, compared to the test runs in the fermenter.

Table 73: Comparison of the xylose degradation rate of the pretests and fermenter runs with Xall-medium and hydrolysate-medium for the yeast strains A027, C238 and C245. The fermenter runs of C238 with hydrolysate-medium were carried out with peptone and yeast extract (B), while C245 was tested without (A).

Experiment	Medium		Δ Xylose [g/L/h]		
			A027	C238	C245
Culture bottles	Xall A		0,06	0,09	0,14
	Xall B		0,06	0,09	0,08
Fermenter	Xall B	Batch	0,08	0,17	0,25
		Feed	0,06	0,27	0,24
Culture bottles	Hydrolysate A		0,01	0,05	0,05
	Hydrolysate B		0,01	0,04	0,05
Fermenter	Hydrolysate A/B	Batch	-	0,15	0,18
		Feed	-	0,19	0,34

A faster xylose degradation was also found at the beginning of the feed-parts in the fermenter runs with Xall-medium, during the higher xylose concentration of around 100 g/L (see chapter 6.2). As described in chapter 3.5.2, the initial xylose concentration is an important factor on the activity of the xylose metabolizing enzymes XR and XDH (McMillan, 1993, Winkelhausen and Kuzmanova, 1998). This means that more xylose can be metabolized when the concentration is high. For a better understanding, further examinations with different xylose concentrations should be done.

Due to the inefficient xylose degradation and/or inefficient formation of ethanol and xylitol, the yeast strains C240 (*Candida utilis*), C255 (*Candida lignohabitans*) and HA1129 (*Candida intermedia*) were sorted out after the pretests in the culture bottles.

Table 74: Comparison of the max. ethanol concentration of the pretests and fermenter runs with Xall-medium and hydrolysate-medium for the yeast strains A027, C238 and C245. The fermenter runs of C238 with hydrolysate-medium were carried out with peptone and yeast extract (B), while C245 was tested without (A).

Experiment	Medium		max. Ethanol [g/L]		
			A027	C238	C245
Culture bottles	Xall A		6,63	4,11	2,11
	Xall B		8,08	5,56	2,26
Fermenter	Xall B	Batch	10,11	4,19	1,44
		Feed	3,02	9,15	0,01
Culture bottles	Hydrolysate A		3,98	5,59	2,79
	Hydrolysate B		4,28	5,93	3,40
Fermenter	Hydrolysate A/B	Batch	-	3,82	2,22
		Feed	-	14,36	13,49

The engineered *S. cerevisiae* strain A027 reached a maximum ethanol yield of 0,29 g ethanol/g sugar and 0,25 g ethanol/g C5 sugars in batch fermenter runs with synthetic Xall-medium B. While the ethanol yield from C5 sugars seemed higher in the pretests with hydrolysate-medium, the xylose degradation was very poor (0,01 g/g), which leads to an inefficient fermentation. The higher ethanol concentration, low xylitol concentration and poor xylose degradation coincides with the results of Novy et al. (2014). This author described the low xylose degradation rate as a mutual problem of genetic modified *S. cerevisiae.*

The unknown yeast strain C238 reached the highest ethanol yields from all sugars in the fermenter runs with hydrolysate-medium B (0,20-0,22 g/g) with a good xylose degradation, but the ethanol yield from C5 sugars was low at 0,06-0,09 g/g (see Table 75).

Having a look on Table 74, the addition of 1 g/L peptone and 0,5 g/L yeast extract seem to have a slightly positive effect on the ethanol formation.

Table 75: Comparison of the max. ethanol yield and ethanol yield of C5 sugars of the pretests and fermenter runs with Xall-medium and hydrolysate-medium for the yeast strains A027, C238 and C245. The fermenter runs of C238 with hydrolysate-medium were carried out with peptone and yeast extract (B), while C245 was tested without (A).

Experiment	Medium		max. Ethanol yield [g ethanol/g sugars]			Ethanol yield [g ethanol /g C5] between 0g/L C6 and MAX		
			A027	C238	C245	A027	C238	C245
Culture bottles	Xall A		0,21	0,10	0,06	0,15	0,03	-
	Xall B		0,22	0,14	0,07	0,16	0,04	0,01
Fermenter	Xall B	Batch	0,29	0,11	0,06	0,25	0,03	-
		Feed	0,25	0,11	-	0,25	0,11	-
Culture bottles	Hydrolysate A		0,27	0,16	0,13	0,44	0,10	-
	Hydrolysate B		0,26	0,21	0,14	0,46	0,17	0,03
Fermenter	Hydrolysate A/B	Batch	-	0,20	0,08	-	0,03	0,01
		Feed	-	0,22	0,11	-	0,09	0,04

Summarized none of the tested yeast strains reached ethanol yields or concentrations high enough for economical usage in synthetic Xall-medium or hydrolysate-medium. Instead the yeast strain C245 and C238 reached high xylitol concentrations and yields.

The highest xylitol concentrations in all experiments were reached by the yeast strain C245. It reached the highest xylitol concentration of all experiments and all yeast strains in the fermenter runs with Xall-medium B (see Table 76). The maximum concentration of 53,2 g/L was measured in the feed-part of the runs. In the pretests, C245 reached between 22,3-22,5 g/L with Xall-medium, 13,4 g/L with hydrolysate-medium A and 6,4 g/L in hydrolysate-medium B with peptone and yeast extract. This means the addition of peptone and yeast extract lowered the xylitol concentration by 50%, while the biomass formation rose up by 50% (e.g. for C245 from 0,12 g CDM/g sugar to 028 g CDM/g sugar). This phenomenon was observed for C245 (*Candida* sp.) and C255 (*Candida lignohabitans).* The fermenter runs in hydrolysate-medium were therefore carried out without peptone and yeast extract. The shift from xylitol formation to biomass production, triggered by these additives, could also be a task of further studies.

As observed in the pretests, the conversion of the xylose was lower in the hydrolysate-medium and C245 only reached a maximum xylitol concentration of 47,2 g/L (Δ feed-parts 39,5 g/L) in the

fermenter run with hydrolysate-medium A. C245 also reached the highest xylitol yield in the culture bottles with the synthetic Xall-medium (0,81-0,83 g xylitol/g xylose). This is very close to the maximum reported xylitol yield of 0,88 g/g, reached in the literature for *Candida guilliermondii* FTI 20037 with wheat straw hydrolysate (Canilha et al., 2006) and *Candida* sp. 11-2 with sugar cane bagasse (Winkelhausen and Kuzmanova, 1998).

Table 76: Comparison of the max. xylitol concentration and xylitol yield of the pretests and fermenter runs with Xall-medium and hydrolysate-medium for the yeast strains A027, C238 and C245. The fermenter runs of C238 with hydrolysate-medium were carried out with peptone and yeast extract (B), while C245 was tested without (A).

Experiment	Medium		max. Xylitol [g/L]			max. Xylitol yield [g xylitol/g xylose]		
			A027	C238	C245	A027	C238	C245
Culture bottles	Xall A		4,96	10,96	22,28	0,23	0,40	0,81
	Xall B		5,84	10,49	22,54	0,24	0,37	0,83
Fermenter	Xall B	Batch	2,28	15,73	20,86	0,11	0,56	0,73
		Feed	0,12	37,92	53,21	0,02	0,49	0,69
Culture bottles	Hydrolysate A		0,16	1,95	13,39	0,06	0,08	0,64
	Hydrolysate B		0,19	1,82	6,35	0,04	0,09	0,31
Fermenter	Hydrolysate A/B	Batch	-	13,56	10,59	-	0,53	0,46
		Feed	-	22,52	39,49	-	0,64	0,46

While C245 was known as xylitol producing yeast strain from the pretests, the unknown yeast strain C238 had a lower xylitol yield (0,37-0,40 g/g) and concentration (10,5-11,0 g/L) in the pretests with the synthetic Xall-medium and was intended for the production of ethanol. In the fermenter runs with Xall-medium, C245 only reached a xylitol yield of 0,60-0,73 g/g (maximum concentration of 70,2 g/L, Δ feed-parts 53,2 g/L), while C238 reached yields of 0,49-0,56 g/g and a xylitol concentration up to 51,0 g/L (Δ feed-part 37,9 g/L). In the hydrolysate-medium runs, C238 only reached a maximum xylitol concentration of 33,2 g/L (Δ feed part 22,5 g/L), which is 42% lower compared to C245. The xylitol yield was in this case 28% higher for C238 compared to C245. The higher xylitol concentration occurred due to the higher xylose degradation rate of C245 compared to C238 (see Table 73).

Nevertheless, beside the maximum xylitol yield mentioned above, *C. guilliermondii* FTI 20037 also reached different xylitol yields with rice straw (0,69 g/g) and sugar cane bagasse (0,48 g/g) (Winkelhausen and Kuzmanova, 1998). The xylitol yield is depended on the yeast strain, oxygen supply, hydrolysate-medium composition and xylose concentration. However, as described in the literature, the best xylitol producers seem to be of the *Candida* genus (see chapter 3.5.2). A sequencing of C238, the currently unknown yeast strain, would be useful to draw conclusions for a better comparison with other references and to find out if it also belongs to the *Candida* genus.

The formation of side-products, like glycerol could also affect the xylitol yield. A high amount of glycerol formed by C245, was measured in the fed-batch fermenter runs with Xall-medium B (see Table 77). The glycerol concentration linearly increased over the whole fermentation time and seemed to be dependent on the formed xylitol concentration. In the fermenter runs with hydrolysate-medium B, the glycerol concentration was lower and only increase at the beginning of the batch-part and after the addition of the sugar feed. Afterwards the concentration was nearly constant over the remaining period. A possible explanation could be the role of glycerol formation, as an important response of the

yeasts on cellular stress situations, like osmotic stress (Flores et al., 2000, Priscila and Felipe, 2008). The dependence of glycerol formation on the glucose or ethanol concentration in the fermenter runs with hydrolysate-medium, could be explained by the increase of osmotic stress during the formation of ethanol. Further research on the dependence of glycerol formation on the oxygen supply, the added sugars and the redox imbalance (see chapter 6.3.2), could help to lower the by-product formation of the yeast strains.

Table 77: Comparison of the max. glycerol concentration of the pretests and fermenter runs with Xall-medium and hydrolysate-medium for the yeast strain C245.

Experiment	Medium		max. Glycerol [g/L]
Culture bottles	Xall A		3,45
	Xall B		2,51
Fermenter	Xall B	Batch	5,72
		Feed	19,46
Culture bottles	Hydrolysate A		4,32
	Hydrolysate B		2,14
Fermenter	Hydrolysate A	Batch	2,95
		Feed	5,88

In summary, none of the executed tests exposed a yeast strain for the efficient ethanol production from wheat straw hydrolysate. The most promising yeast strain to produce ethanol from Xall-medium was A027, but the xylose degradation in hydrolysate-medium was too slow. To improve the fermentation, it should be investigated what kept the engineered *S. cerevisiae* strain from producing ethanol from C5 sugars in the hemicellulose hydrolysate.

The experiments proved, that the conversion of wheat straw hemicellulose hydrolysate to xylitol is more useful than the conversion to ethanol. The yeast strain C245 reached the highest xylitol concentrations in the fermenter runs, the highest xylitol yields with Xall-medium B in the culture bottles and in the fermenter runs (see Table 76). The highest xylitol yields in the fermenter runs with hydrolysate-medium were reached by C238 (0,46 g/g).

For a high xylitol concentration, a limited oxygen supply was maintained. The oxygen supply is one of the most important parameters in the fermentation and degradation of xylose and arabinose, and the formation of products. Further experiments could reveal the mechanism of oxygen on the xylose degradation and the respective optimum oxygen input for the preferred product with each yeast strain. On the metabolic level, the metabolic flux analysis (e.g. using isotopically labeled sugars 13C) of the yeast strains would be helpful, to find out how the sugars were converted into the different products.

Overall, it can be said that a lot of research still needs to be done in order to efficiently produce ethanol and xylitol from hemicellulose hydrolysate in a large scale. Especially the full understanding of the metabolic background of the different yeast strains would greatly help to optimize the process. The efficient utilization and bioconversion of raw materials will be even more important for future generations. It can be expected, that the interest in the research on new microorganisms and the implementation of new processes for the production of valuable chemicals on the basis of waste materials, will further increase in the future. Hopefully, this thesis will have a positive impact on future researches and developments dealing with this scientific subject.

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9 Appendix

9.1 Chemicals

Appendix table 1: List of chemicals used for media or HPLC-standards

Chemicals	Manufacturer
4-Aminobenzoic acid	Merck
Antifoam agent "Glanapon 2000"	Bussetti & Co
Calcium dichloride dihydrate	Roth
Calcium-D(+)-panthothenate	Merck
Cobalt chloride hexahydrate	Merck
Copper(II) sulfate pentahydrate	Merck
D(-)-Mannitol	Merck
D(+)-Arabitol	Roth
D(+)-Biotin	Roth
D(+)-Galactose	Roth
D(+)-Glucose anhydrous	Roth
D(+)-Mannose	Roth
D(+)-Xylose	Roth
Diammonium hydrogen phosphate	Roth
Dipotassium hydrogen phosphate	Merck
Ethanol abs.	Riedel-deHaen
Glycerol	Roth
Hydrochloric acid solution 32%	Roth
Iron (II) sulfate	Merck
L(+)-Arabinose	Roth
Magnesium sulfate heptahydrate	Roth
Manganese(II) sulfate monohydrate	Merck
meso-Inositol	Roth
Nicotinic acid	n.a.
Pyridoxol hydrochloride	Merck
Sodium acetate anhydrous	Merck
Sodium chloride	Roth
Sodium hydroxide granulate	Roth
Thiamine hydrochloride	Stettfurt 6/85
Tryptone/Peptone ex casein granulated	Roth
Xylitol	Merck
Yeast extract granulated	Merck
Zinc sulfate heptahydrate	Merck

9.2 Equipment

Appendix table 2: List of technical equipment of the experiments

Technical equipment	Manufacturer	Designation
Acu pipet aid	IBS Biosciences	Pipetboy
Analytical balance	Sartorius analytics	A210P
Autoclave 18 liter	CertoClave	Classic 125°C/140°C
Autoclave 80 L	JP Selecta, s.a.	Presoclave II 80
Balance 1 (Chem. Laboratory)	Sartorius	LC6201S
Balance 2 (Microbiology)	Sartorius	LP6200S
Centrifuge	Sigma	6-10
Centrifuge (table top)	Hettich	Rotofix 32
Circular filter paper (filtration hydrolysate)	Macherey-Nagel	Ashless circles MN640w 18,5 cm
Compartment drier 105°C	Heraeus	T5042
Compartment drier 180°C	Köttermann	2713
Cooking pots (without lid)	Certoclav	Typ CV 2
Cuvettes	Roth Rotilab	Single-use cells, PS 1,6 mL, XK20
Deep freezer -20°C	Liebherr	GSP 3626
Filter (for dry weight determination)	GE Healthcare	Whatman Glass Microfiber Filters GF/C d=47mm
HPLC	Shimadzu Corp.	*see chapter 4.4.5*
HPLC caps for vials	Roth	Screw caps with bore hole, PP, ND 8, KE 35.1
HPLC vials	Roth Rotilab	Sample vials 2 mL, E159.1
Incubator (test bottles)	Köttermann	2737
Incubator shaker (pre-culture)	Infors HAT	RS-1T
Laminar flow work bench	Gelaire	Air Flow Class 100
Magnetic stirrer	Janke & Kunkel	KMO2
Magnetic stirrer (test bottles)	Variomag	Multipoint 6 / Mulitpoint 15
Microscope	Micros Austria GmbH	MC 400
Mill (straw coarse grinding)	Waring	Commercial Blender
Mill (straw fine grinding)	Retsch	SR2, mesh sieve = 0,75 mm
Objective 1	Micros Austria GmbH	Plan 40/0,65
Objective 2	Micros Austria GmbH	Plan 60/0,85
Objective 3	Micros Austria GmbH	Plan 100/1,25
Peristaltic pump (Air-input fermenter)	Gilson	Minipuls 3
Peristaltic pump tubing	Elkay Élreann	Standard Tubing, purple/orange Inner diameter= 0,1 inches
pH electrode	Schott	H61
Photometer	Shimadzu	UV-1601
Pipettes	Sartorius	mLine
Rotavapor	Büchi	R-205
Rotavapor heating bath	Büchi	B-490
Rotavapor vacuum controller	Büchi	V-800
Ultra-low temperature freezer 80°C	Sanyo	Ultra low MDF-192
Ultrasonic bath	Bandelin	Sonorex Transitor
Vortex	Merck	Eurolab
Water bath & Recirculation (fermenter, old)	MgW Lauda	C20 & D8-L
Water bath (fermenter, new)	Huber	Polystat CC2

9.3 Results of the examination of micro-aeration rate in the fermenter

Appendix table 3: Aeration rate results of gas supply experiment with 20 rpm feeding-pump and stirring stage 3

air volume start [mL]	air volume end [mL]	Δ air volume [mL]	Δ duration [min]	aeration rate [mL /min]
40	80	40	10,5	3,81
40	100	60	15,48	3,88
42	80	38	9,25	4,11
42	100	58	14,55	3,99
40	80	40	10,04	3,98
40	100	60	15,32	3,92
		Ø aeration rate [mL /min]		**3,95**
		Ø aeration rate [vvm]		**0,004**

Appendix table 4: Aeration rate results of gas supply experiment with 40 rpm feeding-pump and stirring stage 3

air volume start [mL]	air volume end [mL]	Δ air volume [mL]	Δ duration [min]	aeration rate [mL /min]
45	80	35	3,15	11,11
45	100	55	5,18	10,62
40	80	40	3,43	11,66
40	100	60	5,41	11,09
40	80	40	3,4	11,76
40	100	60	5,38	11,15
40	80	40	3,42	11,70
40	100	60	5,39	11,13
		Ø aeration rate [mL /min]		**11,28**
		Ø aeration rate [vvm]		**0,011**

Appendix table 5: Air bubbles results of gas supply experiment with 20 rpm feeding-pump and stirring stage 3

air bubbles [ ]	Δ duration [min]	air bubbles [1/min]
44	9,2	4,8
63	14,49	4,3
40	8,25	4,8
62	13,55	4,6
41	9,04	4,5
60	14,32	4,2
	Ø air bubbles [1/min]	**4,55**

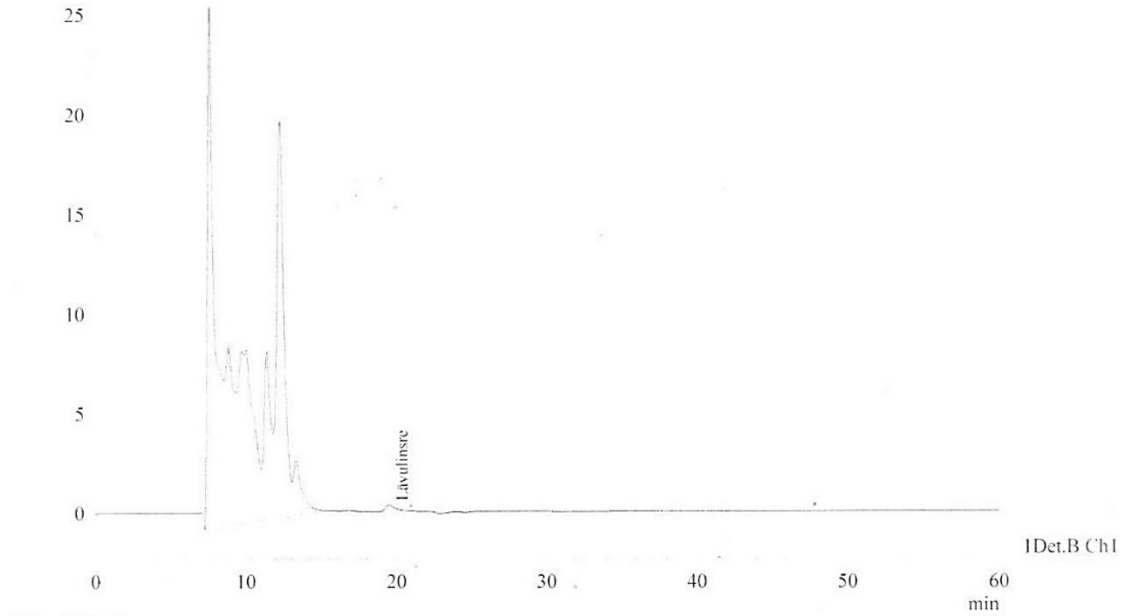
9.4 Examination of inhibitors in the hydrolysate

Sample Information

Acquired by	: Admin
Sample Name	: Hydrolysat 24.11.15 1:100
Sample ID	: Wdh
Tray#	: 1
Vail#	: 2
Injection Volume	: 20 uL
Data Filename	: 21.01.2016_Hydrolysat 24.11.15 1$100_Wdh_2.lcd
Method Filename	: Xyl-Inhibitors_20160121.lcm
Batch Filename	: 20160121_Inhibitoren+STD Xall_HPLC1.lcb
Report Filename	: Report Format_pollak.lcr
Date Acquired	: 21.01.2016 15:26:53
Data Processed	: 21.01.2016 16:26:55

Chromatogram

Hydrolysat 24.11.15 1:100 D:\HPLC\2015\VeC\HPLC1\Inhibitoren Methode\21.01.2016_Hydrolysat 24.11.15 1$100_Wdh_2.lcd



1 Det.B Ch1 /

Quantitative Results

Detector B

ID#	Name	Ret. Time	Area	Height	Conc.
1	Milchsäure	0.00	0	0	0.00
2	Glycerin	0.00	0	0	0.00
3	Ameisensäure	0.00	0	0	0.00
4	Essigsäure	0.00	0	0	0.00
5	Lävulinsre	19.47	12106	315	0.05
6	Ethanol	0.00	0	0	0.00
7	HMF	0.00	0	0	0.00
8	Furfural	0.00	0	0	0.00
9	2,3-Butandiol	0.00	0	0	0.00

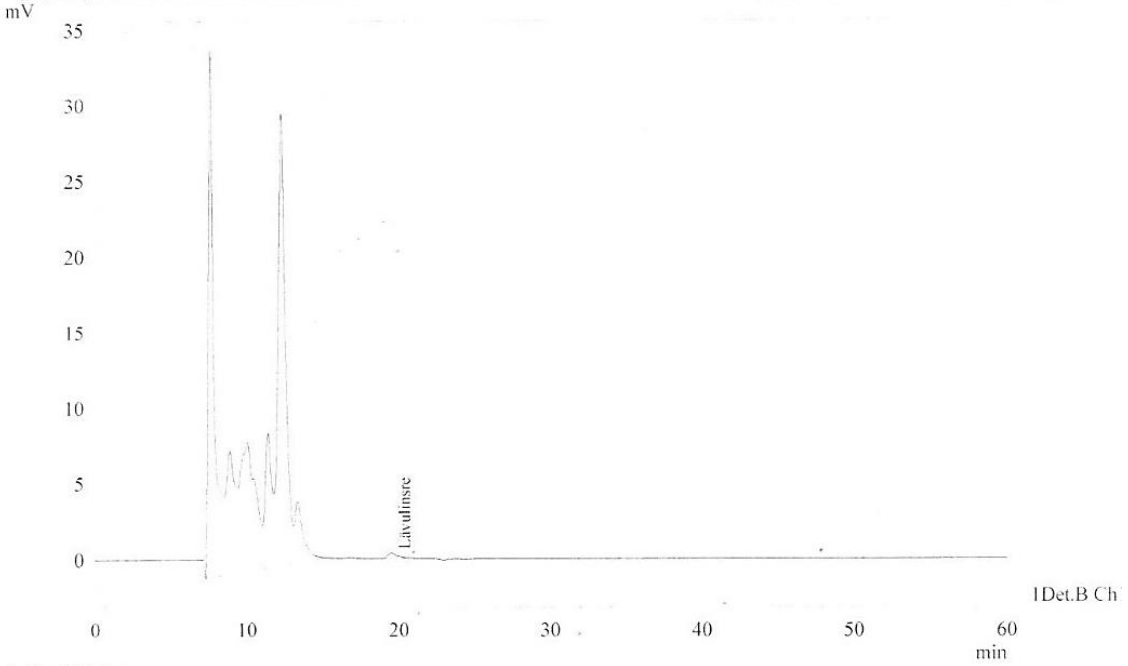
Appendix figure 1: Raw data of the inhibitor measurement with HPLC – Hydrolysate batch 24.11.2015 used for the hydrolysate-medium pretests in the culture bottles

Sample Information

Acquired by	: Admin
Sample Name	: Hydrolysat 11.01.16 1:100
Sample ID	: Wdh
Tray#	: 1
Vail#	: 3
Injection Volume	: 20 uL
Data Filename	: 21.01.2016_Hydrolysat 11.01.16 1$100_Wdh_3.lcd
Method Filename	: Xyl-Inhibitors_20160121.lcm
Batch Filename	: 20160121_Inhibitoren+STD Xall_HPLC1.lcb
Report Filename	: Report Format_pollak.lcr
Date Acquired	: 21.01.2016 16:27:17
Data Processed	: 21.01.2016 17:27:19

Chromatogram

Hydrolysat 11.01.16 1:100 D:\HPLC\2015\VeC\HPLC1\Inhibitoren Methode\21.01.2016_Hydrolysat 11.01.16 1$100_Wdh_3.lcd



1 Det.B Ch1 /

Quantitative Results

Detector B

ID#	Name	Ret. Time	Area	Height	Conc.
1	Milchsäure	0.00	0	0	0.00
2	Glycerin	0.00	0	0	0.00
3	Ameisensäure	0.00	0	0	0.00
4	Essigsäure	0.00	0	0	0.00
5	Lävulinsre	19.48	13535	343	0.05
6	Ethanol	0.00	0	0	0.00
7	HMF	0.00	0	0	0.00
8	Furfural	0.00	0	0	0.00
9	2,3-Butandiol	0.00	0	0	0.00

Appendix figure 2: Raw data of the inhibitor measurement with HPLC – Hydrolysate batch 11.01.2016 used for the fermenter runs with hydrolysate-medium of C238 and C245

9.5 Comparison of the varied fermenter runs of C245/ *Candida* sp.

Appendix table 6: Yeast strain C245 in the fermenter with Xall-medium A, B and with 3-folded inoculum in a fed-batch process. Overview of the percentage of consumed sugars at the end of the experiment

Process	Medium	Δ Run duration [h]	Δ Xylose End [%]	Δ Xylose [g/L/h]	Δ Arabinose End [%]	Δ Arabinose [g/L/h]	Δ C6 sugars 100 h [%]
Batch	Xall B	123,3	97,8	0,24	11,0	0,003	100,0
		114,0	97,8	0,25	35,9	0,012	
Feed		333,5	100,0	0,27	52,8	0,018	
		333,5	80,1	0,21	20,8	0,007	
Batch	Xall A	142,8	100,0	0,20	44,8	0,009	
Feed		333,8	100,0	0,27	47,6	0,017	
3-fold Inoc. Batch		71,3	83,4	0,34	11,7	0,005	
3-fold Inoc. Feed		290,3	90,8	0,31	25,9	0,011	

Appendix table 7: Yeast strain C245 in the fermenter with Xall-medium A, B and with 3-folded inoculum in a fed-batch process. Overview of the OD_{600}, CDM [g/L/OD], CDM at the end of the cultivation and the biomass yield.

Process	Medium	Δ Run duration [h]	OD_{600} End	CDM [g/L/OD]	CDM End [g/L]	Biomass yield [g CDM/g sugar]
Batch	Xall B	123,25	4,23	0,46	1,93	0,05
		114	4,78	0,26	1,23	0,03
Feed		333,5	7,66	0,31	2,41	0,01
		333,5	7,21	0,28	2,05	0,01
Batch	Xall A	142,75	5,39	0,32	1,73	0,04
Feed		333,75	6,76	0,21	1,43	0,00
3-fold Inoc. Batch		71,25	3,36	0,30	1,01	0,03
3-fold Inoc. Feed		290,25	5,01	0,24	1,20	0,00

Appendix table 8: Yeast strain C245 in the fermenter with Xall-medium A, B and with 3-folded inoculum in a fed-batch process. Overview of the pH decrease at the end of the fermentation.

Process	Medium	Δ Run duration [h]	Δ pH End
Batch	Xall B	123,25	-0,57
		114	-0,29
Feed		333,5	-0,78
		333,5	-0,59
Batch	Xall A	142,75	-0,83
Feed		333,75	-2,29
3-fold Inoc. Batch		71,25	-0,30
3-fold Inoc. Feed		290,25	-0,99

Appendix table 9: Yeast strain C245 in the fermenter with Xall-medium A, B and with 3-folded inoculum in a fed-batch process. Maximum produced concentrations of the desired product ethanol and the ethanol yield.

Process	Medium	Δ Run duration [h]	Δ max. Ethanol [g/L]	max. Ethanol [g/L]	max. Ethanol yield [g EtOH /g sugar]
Batch	Xall B	123,25	1,35	1,35	0,05
		114	1,53	1,53	0,06
Feed		333,5	0,00	0,98	0,00
		333,5	0,01	1,22	0,00
Batch	Xall A	142,75	1,00	1,00	0,05
Feed		333,75	0,28	0,99	0,01
3-fold Inoc. Batch		71,25	1,34	1,34	0,10
3-fold Inoc. Feed		290,25	0,00	0,98	0,00

Appendix table 10: Yeast strain C245 in the fermenter with Xall-medium A, B (with peptone and yeast extract) and with 3-folded inoculum in a fed-batch process. Maximum produced concentrations of the desired product xylitol and the xylitol yield.

Process	Medium	Δ Run duration [h]	Δ max. Xylitol [g/L]	max. Xylitol [g/L]	max. Xylitol yield [g Xylitol /g Xylose]
Batch	Xall B	123,25	20,77	20,77	0,70
		114	20,94	20,94	0,76
Feed		333,5	56,58	73,73	0,66
		333,5	49,84	66,75	0,71
Batch	Xall A	142,75	18,22	18,22	0,64
Feed		333,75	63,59	78,14	0,71
3-fold Inoc. Batch		71,25	20,05	20,05	0,82
3-fold Inoc. Feed		290,25	58,01	74,68	0,66

Appendix table 11: Yeast strain C245 in the fermenter with Xall-medium A, B (with peptone and yeast extract) and with 3-folded inoculum in a fed-batch process. Maximum produced concentrations of ethanol, xylitol, acetic acid, glycerol and arabitol/mannitol.

Process	Medium	Δ Run duration [h]	max. Ethanol [g/L]	max. Xylitol [g/L]	max. Acetic acid [g/L]	max. Glycerol [g/L]	max. Arabitol/ Mannitol [g/L]
Batch	Xall B	123,25	1,35	20,77	0,00	5,91	0,41
		114	1,53	20,94	0,00	5,52	0,38
Feed		333,5	0,98	73,73	0,00	23,38	6,47
		333,5	1,22	66,75	0,00	15,53	3,15
Batch	Xall A	142,75	1,00	18,22	0,00	6,65	1,13
Feed		333,75	0,99	78,14	0,00	17,70	7,12
3-fold Inoc. Batch		71,25	1,34	20,05	0,00	2,82	0,31
3-fold Inoc. Feed		290,25	0,98	74,68	0,00	16,94	3,22

9.6 Raw data of the cultivations in culture bottles with Xall-medium

Appendix table 12: A027 Xall-medium A (run 1)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,13	0,073	0,00	0,00	29,05	2,90	4,30	3,48	4,35	0,00	0,00	0,00
1	6,11	0,642	0,00	0,00	28,67	2,57	3,93	3,43	4,23	0,00	0,00	0,00
18	5,65	4,989	3,46	0,35	25,53	0,00	2,38	3,24	0,35	0,17	0,93	0,00
42	5,46	6,694	4,01	1,03	23,26	0,00	2,10	2,88	0,22	0,34	1,12	0,40
66	5,30	7,164	4,56	1,33	22,07	0,00	0,00	2,36	0,00	0,40	1,20	0,60
138	4,99	9,928	5,85	1,62	16,32	0,00	0,00	1,95	0,00	0,44	1,33	0,86
186	4,81	10,744	6,39	1,80	13,54	0,00	0,00	1,88	0,00	0,41	1,39	0,97
234	4,59	11,500	6,59	2,06	11,50	0,00	0,00	1,75	0,00	0,35	1,45	1,09
306	4,22	12,608	6,47	2,29	8,08	0,00	0,00	1,83	0,00	0,15	1,48	1,15
354	3,58	15,236	6,08	2,64	5,28	0,00	0,00	1,74	0,00	0,00	1,48	1,18
402	2,96	18,780	5,65	4,36	2,12	0,00	0,00	1,82	0,00	0,00	1,54	1,73
497	2,73	25,960	4,33	1,76	0,00	0,00	0,00	0,10	0,00	0,00	0,98	0,64
497	2,71	25,960	CDM =7,02g/L				0,270	g CDM/L/OD				

Appendix table 13: A027 Xall-medium A (run 2)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,13	0,073	0,00	0,00	29,05	2,90	4,30	3,48	4,35	0,00	0,00	0,00
1	6,10	0,653	0,00	0,00	28,79	2,57	4,05	3,46	4,25	0,00	0,00	0,00
18	5,65	4,960	3,43	0,34	25,56	0,00	2,36	3,24	0,35	0,18	0,96	0,00
42	5,49	6,132	3,91	1,16	23,11	0,00	2,01	2,81	0,23	0,33	1,16	0,36
66	5,27	6,162	4,35	1,76	22,45	0,00	0,00	2,47	0,10	0,38	1,29	0,58
138	5,00	6,514	5,61	2,58	17,90	0,00	0,00	1,98	0,00	0,55	1,54	0,99
186	4,99	7,912	6,16	2,78	16,05	0,00	0,00	1,93	0,00	0,53	1,62	1,11
234	4,79	8,052	6,32	2,76	13,72	0,00	0,00	1,57	0,00	0,56	1,66	1,19
306	4,63	9,264	6,38	2,94	11,26	0,00	0,00	1,52	0,00	0,45	1,70	1,29
354	4,76	9,228	6,33	3,21	9,93	0,00	0,00	1,33	0,00	0,35	1,71	1,34
402	4,81	9,388	6,67	5,56	12,84	0,00	0,00	1,94	0,00	0,22	1,86	2,01
497	3,32	15,104	6,38	4,62	0,91	0,00	0,00	1,07	0,00	0,00	1,80	1,44
497	3,30	15,104	CDM =3,96g/L				0,262	g CDM/L/OD				

Appendix table 14: A027 Xall-medium B (run 1)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,13	0,079	0,00	0,00	28,39	2,84	4,07	3,38	4,29	0,00	0,00	0,00
1	6,11	0,765	2,02	0,00	28,03	2,39	4,00	3,33	3,12	0,10	0,29	0,00
18	5,72	5,760	3,71	0,58	24,58	0,00	2,27	3,12	0,31	0,26	0,72	0,12
42	5,54	7,268	4,40	1,73	21,80	0,00	1,83	2,63	0,19	0,48	0,88	0,46
66	5,37	7,444	4,99	2,39	20,79	0,00	0,00	2,32	0,07	0,57	0,98	0,71
138	5,07	9,068	6,56	3,44	14,56	0,00	0,00	1,68	0,00	0,69	1,20	1,12
186	5,07	9,096	7,31	3,61	12,40	0,00	0,00	1,61	0,00	0,70	1,27	1,26
234	4,91	9,344	7,75	3,75	10,51	0,00	0,00	1,39	0,00	0,67	1,33	1,41
306	4,94	9,868	8,07	3,95	7,80	0,00	0,00	1,27	0,00	0,56	1,40	1,50
354	5,04	9,784	7,96	4,24	6,19	0,00	0,00	1,26	0,00	0,42	1,39	1,57
402	5,08	10,308	8,06	7,48	5,91	0,00	0,00	1,48	0,00	0,28	1,52	2,33
497	5,12	12,660	5,70	6,00	0,00	0,00	0,00	0,68	0,00	0,00	1,18	1,66
497	5,12	12,660	CDM =3,08 g/L			0,243	g CDM/L/OD					

Appendix table 15: A027 Xall-medium B (run 2)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,13	0,079	0,00	0,00	28,39	2,84	4,07	3,38	4,29	0,00	0,00	0,00
1	6,10	0,776	0,00	0,00	28,28	2,50	4,13	3,41	4,24	0,00	0,00	0,00
18	5,71	5,659	3,65	0,55	24,89	0,00	2,20	3,16	0,36	0,25	0,78	0,00
42	5,50	7,474	4,29	1,52	22,15	0,00	2,04	2,75	0,24	0,48	0,99	0,44
66	5,34	7,940	4,95	1,99	20,58	0,00	0,00	2,32	0,07	0,59	1,11	0,65
138	4,95	10,296	6,38	2,41	15,20	0,00	0,00	1,95	0,00	0,68	1,32	0,96
186	4,95	10,656	7,10	2,33	11,75	0,00	0,00	1,72	0,00	0,63	1,43	1,03
234	4,80	10,908	7,46	2,53	9,70	0,00	0,00	1,74	0,00	0,55	1,49	1,15
306	4,65	12,884	7,41	2,69	6,08	0,00	0,00	1,66	0,00	0,33	1,54	1,24
354	4,54	13,896	7,16	2,85	4,05	0,00	0,00	1,63	0,00	0,18	1,55	1,32
402	3,96	15,880	8,08	4,19	1,14	0,00	0,00	1,34	0,00	0,00	1,40	1,74
497	3,30	24,360	7,07	1,84	0,00	0,00	0,00	0,00	0,00	0,00	1,62	0,89
497	3,27	24,360	CDM =6,46 g/L			0,265	g CDM/L/OD					

Appendix table 16: C238 Xall-medium A (run 1)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,00	0,075	0,00	0,00	29,90	3,06	3,20	3,24	3,36	0,00	0,00	0,00
3	5,96	0,934	0,49	0,00	29,37	1,62	3,15	3,19	3,36	0,00	0,07	0,00
20	5,73	4,673	2,93	2,96	25,02	0,00	1,70	3,20	0,00	0,00	0,64	0,00
44	5,57	5,496	3,45	5,20	21,85	0,00	0,00	3,13	0,00	0,00	0,67	0,11
68	5,33	7,814	3,38	6,78	17,45	0,00	0,00	2,82	0,00	0,00	0,44	0,19
140	4,15	14,350	3,84	10,63	8,04	0,00	0,00	2,40	0,00	0,06	0,53	0,45
188	3,47	15,935	3,95	11,32	3,99	0,00	0,00	2,12	0,00	0,00	0,30	0,55
236	3,20	19,560	3,95	11,45	1,20	0,00	0,00	1,88	0,00	0,00	0,08	0,90
308	2,73	25,045	1,91	10,58	0,00	0,00	0,00	2,10	0,00	0,00	0,00	1,21
356	2,71	31,384	0,12	10,34	0,00	0,00	0,00	2,10	0,00	0,00	0,00	1,29
476,5	2,86	35,408	0,00	9,73	0,00	0,00	0,00	1,65	0,00	0,00	0,00	1,35
476,5	2,85	35,408	CDM = 7,54 g/L			0,213	g CDM/L/OD					

Appendix table 17: C238 Xall-medium A (run 2)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,00	0,075	0,00	0,00	29,90	3,06	3,20	3,24	3,36	0,00	0,00	0,00
3	5,96	0,999	0,45	0,00	29,09	1,60	3,04	3,18	3,32	0,00	0,07	0,00
20	5,72	4,817	2,97	3,26	24,24	0,00	1,30	3,14	0,00	0,00	0,65	0,00
44	5,54	5,759	3,40	5,34	21,03	0,00	0,00	3,14	0,00	0,00	0,70	0,00
68	5,35	7,686	3,39	6,27	17,85	0,00	0,00	2,87	0,00	0,00	0,65	0,20
140	4,43	13,125	3,79	9,32	10,53	0,00	0,00	2,48	0,00	0,19	0,70	0,40
188	3,80	14,375	4,01	10,09	6,73	0,00	0,00	2,15	0,00	0,00	0,45	0,48
236	3,43	17,220	4,28	10,47	3,72	0,00	0,00	2,06	0,00	0,00	0,33	0,63
308	2,96	22,850	4,14	10,26	0,65	0,00	0,00	2,41	0,00	0,00	0,00	0,87
356	2,73	27,872	2,70	9,82	0,00	0,00	0,00	2,28	0,00	0,00	0,00	1,00
476,5	2,87	36,632	0,00	9,56	0,00	0,00	0,00	2,29	0,00	0,00	0,00	1,25
476,5	2,88	36,632	CDM = 7,82 g/L			0,213	g CDM/L/OD					

Appendix table 18: C238 Xall-medium B (run 1)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,01	0,081	0,00	0,00	29,16	5,86	3,09	3,16	3,28	0,00	0,00	0,00
3	5,90	1,512	1,31	0,00	29,08	2,64	3,11	3,18	3,61	0,00	0,09	0,00
20	5,73	5,947	4,72	3,90	22,61	0,00	0,29	3,12	0,00	0,00	0,50	0,00
44	5,46	8,596	4,86	5,19	19,52	0,00	0,00	2,86	0,00	0,19	0,49	0,18
68	5,22	10,950	4,89	6,09	15,97	0,00	0,00	2,65	0,00	0,22	0,41	0,27
140	4,48	18,010	5,53	9,92	6,11	0,00	0,00	2,17	0,00	0,13	0,24	0,64
188	4,04	18,345	5,60	10,65	2,11	0,00	0,00	1,76	0,00	0,08	0,14	0,75
236	3,66	21,360	5,19	10,89	0,43	0,00	0,00	1,87	0,00	0,00	0,00	1,04
308	3,18	25,575	2,85	9,68	0,00	0,00	0,00	1,77	0,00	0,00	0,00	1,56
356	2,92	31,224	0,11	9,41	0,00	0,00	0,00	1,66	0,00	0,00	0,00	1,66
476,5	3,06	35,528	0,00	7,90	0,00	0,00	0,00	0,45	0,00	0,00	0,00	1,93
476,5	3,06	35,528	CDM = 7,88 g/L				0,222	g CDM/L/OD				

Appendix table 19: C238 Xall-medium B (run 2)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,01	0,081	0,00	0,00	29,16	5,86	3,09	3,16	3,28	0,00	0,00	0,00
3	5,90	1,652	1,34	0,00	28,88	2,57	2,98	3,15	3,59	0,00	0,10	0,00
20	5,73	6,213	4,70	3,67	23,08	0,00	0,23	2,95	0,00	0,00	0,48	0,00
44	5,52	8,222	4,77	4,89	20,20	0,00	0,00	3,00	0,00	0,16	0,45	0,19
68	5,28	10,604	4,67	5,69	16,46	0,00	0,00	2,63	0,00	0,15	0,40	0,29
140	4,63	17,060	5,28	8,83	8,06	0,00	0,00	2,20	0,00	0,18	0,24	0,55
188	4,37	16,820	5,46	9,65	4,09	0,00	0,00	1,84	0,00	0,08	0,20	0,75
236	4,10	19,665	5,53	10,09	1,62	0,00	0,00	2,00	0,00	0,00	0,02	0,96
308	3,41	24,805	3,98	8,98	0,00	0,00	0,00	1,95	0,00	0,00	0,00	1,44
356	3,03	31,112	2,03	8,79	0,00	0,00	0,00	1,65	0,00	0,00	0,00	1,64
476,5	3,03	37,288	0,00	8,27	0,00	0,00	0,00	2,04	0,00	0,00	0,00	1,72
476,5	2,88	37,288	CDM = 8,20 g/L				0,220	g CDM/L/OD				

Appendix table 20: C240 Xall-medium A (run 1)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,97	0,074	0,00	0,00	28,51	2,76	3,50	2,86	3,30	0,00	0,00	0,00
2	5,95	0,591	0,00	0,00	28,08	2,22	3,26	2,85	3,33	0,00	0,00	0,00
17	5,77	2,875	2,07	2,48	24,71	0,00	2,39	2,90	0,00	0,00	0,58	0,00
41	5,69	3,114	2,73	6,11	22,52	0,00	0,00	2,83	0,00	0,00	0,80	0,00
65	5,66	2,970	2,95	8,56	18,74	0,00	0,00	2,80	0,00	0,00	0,96	0,00
137	5,75	4,488	2,90	14,09	11,68	0,00	0,00	2,63	0,00	0,00	1,18	0,21
185	5,51	4,688	2,83	16,79	7,28	0,00	0,00	2,57	0,00	0,00	1,46	0,25
233	5,53	4,484	2,75	18,76	3,43	0,00	0,00	2,86	0,00	0,00	1,62	0,34
305	5,41	5,144	2,52	20,50	0,00	0,00	0,00	2,54	0,00	0,00	2,26	0,59
353	5,33	7,338	2,06	19,56	0,00	0,00	0,00	2,30	0,00	0,00	2,20	0,80
353	2,91	7,338	CDM = 2,06 g/L			0,281	g CDM/L/OD					

Appendix table 21: C240 Xall-medium A (run 2)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,97	0,074	0,00	0,00	28,51	2,76	3,50	2,86	3,30	0,00	0,00	0,00
2	5,94	0,776	0,00	0,00	27,69	2,20	3,67	2,85	3,29	0,00	0,00	0,00
17	5,75	2,964	2,05	2,58	25,10	0,00	2,47	3,00	0,00	0,00	0,59	0,00
41	5,70	3,485	2,74	6,93	21,58	0,00	0,00	2,83	0,00	0,00	0,86	0,00
65	5,63	3,304	2,75	9,69	17,24	0,00	0,00	2,78	0,00	0,00	0,99	0,00
137	5,67	4,678	2,71	14,01	10,57	0,00	0,00	2,73	0,00	0,00	1,24	0,21
185	5,47	4,196	2,69	16,42	7,21	0,00	0,00	2,70	0,00	0,00	1,27	0,24
233	5,41	4,560	2,66	17,68	4,60	0,00	0,00	2,74	0,00	0,00	1,38	0,32
305	5,25	5,544	2,41	19,40	1,41	0,00	0,00	2,68	0,00	0,00	1,75	0,45
353	5,04	7,238	2,09	19,30	0,00	0,00	0,00	2,64	0,00	0,00	1,93	0,66
353	2,91	7,238	CDM = 1,86 g/L			0,257	g CDM/L/OD					

Appendix table 22: C240 Xall-medium B (run 1)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,97	0,076	0,00	0,00	27,90	2,72	3,20	2,82	3,26	0,00	0,00	0,00
2	5,96	0,877	0,20	0,00	27,63	2,06	3,15	2,84	3,15	0,00	0,00	0,00
17	5,81	3,364	2,54	2,76	24,65	0,00	2,09	3,00	0,18	0,00	0,53	0,00
41	5,77	3,721	3,24	6,28	21,84	0,00	0,00	2,83	0,00	0,00	0,72	0,00
65	5,76	3,701	3,13	7,14	19,92	0,00	0,00	2,70	0,00	0,00	0,68	0,00
137	5,81	6,394	3,14	10,38	14,68	0,00	0,00	2,66	0,00	0,00	0,74	0,19
185	5,59	6,464	3,19	11,26	12,51	0,00	0,00	2,46	0,00	0,00	0,76	0,24
233	5,51	7,282	3,18	11,65	10,22	0,00	0,00	2,67	0,00	0,00	0,78	0,32
305	5,40	8,040	3,17	12,59	7,27	0,00	0,00	2,55	0,00	0,00	0,81	0,41
353	5,32	9,436	3,18	13,78	4,73	0,00	0,00	2,43	0,00	0,00	0,81	0,50
353	2,91	9,436	CDM = 2,80 g/L			0,297	g CDM/L/OD					

Appendix table 23: C240 Xall-medium B (run 2)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,97	0,076	0,00	0,00	27,90	2,72	3,20	2,82	3,26	0,00	0,00	0,00
2	5,96	0,903	0,24	0,00	27,42	2,04	3,53	2,83	3,14	0,00	0,00	0,00
17	5,81	3,357	2,59	2,92	24,30	0,00	2,00	2,99	0,14	0,00	0,55	0,00
41	5,77	3,827	3,20	6,62	21,27	0,00	0,00	2,82	0,00	0,00	0,71	0,00
65	5,73	3,661	3,16	7,87	18,99	0,00	0,00	2,78	0,00	0,00	0,70	0,00
137	5,76	5,654	3,03	13,91	10,91	0,00	0,00	2,68	0,00	0,00	0,84	0,24
185	5,58	5,578	3,09	16,52	7,10	0,00	0,00	2,64	0,00	0,00	0,93	0,30
233	5,52	5,886	3,05	17,86	4,25	0,00	0,00	2,60	0,00	0,00	1,04	0,38
305	5,40	6,704	2,86	18,58	1,44	0,00	0,00	2,61	0,00	0,00	1,48	0,53
353	5,32	4,476	2,77	18,31	0,37	0,00	0,00	2,60	0,00	0,00	1,62	0,62
353	2,91	4,476	CDM = 1,86g/L			0,416	g CDM/L/OD					

Appendix table 24: C245 Xall-medium A (run 1)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,97	0,074	0,00	0,00	28,51	2,76	3,50	2,86	3,30	0,00	0,00	0,00
2	5,93	0,562	0,00	0,00	27,59	2,30	3,53	2,85	3,26	0,00	0,00	0,00
17	5,75	2,273	1,39	4,13	23,51	0,00	3,14	2,88	1,67	0,00	0,72	0,00
41	5,64	2,467	1,79	11,69	14,72	0,00	2,32	2,74	0,14	0,00	1,25	0,11
65	5,61	2,454	1,89	16,82	9,21	0,00	0,99	2,41	0,00	0,00	1,59	0,26
137	5,63	3,493	1,97	22,59	1,17	0,00	0,00	2,68	0,00	0,00	2,93	0,51
185	5,47	3,574	1,95	22,11	0,00	0,00	0,00	2,65	0,00	0,00	3,50	0,78
233	5,48	4,191	1,83	20,94	0,00	0,00	0,00	2,03	0,00	0,00	3,86	1,32
305	5,31	5,878	1,53	19,14	0,00	0,00	0,00	1,50	0,00	0,00	4,39	1,72
353	5,29	6,694	1,24	17,93	0,00	0,00	0,00	1,42	0,00	0,00	4,69	1,84
353	2,91	6,694	CDM = 1,92 g/L				0,287	g CDM/L/OD				

Appendix table 25: C245 Xall-medium A (run 2)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,97	0,074	0,00	0,00	28,51	2,76	3,50	2,86	3,30	0,00	0,00	0,00
2	5,93	0,563	0,00	0,00	27,72	2,29	3,31	2,83	3,30	0,00	0,04	0,00
17	5,77	2,128	1,31	3,80	23,10	0,00	3,66	2,80	1,81	0,00	0,68	0,00
41	5,64	2,198	1,75	8,91	18,08	0,00	2,97	2,83	0,23	0,00	0,94	0,00
65	5,67	2,195	1,91	11,21	15,87	0,00	1,87	2,58	0,00	0,00	1,05	0,19
137	5,71	2,713	2,12	15,71	10,81	0,00	0,63	2,37	0,00	0,00	1,28	0,37
185	5,50	2,637	2,24	17,65	7,71	0,00	0,15	2,21	0,00	0,00	1,41	0,45
233	5,53	2,692	2,25	19,43	5,21	0,00	0,00	2,55	0,00	0,00	1,66	0,54
305	5,58	3,004	2,08	20,91	2,38	0,00	0,00	2,57	0,00	0,00	1,83	0,68
353	5,61	4,546	2,05	21,97	0,99	0,00	0,00	2,44	0,00	0,00	2,21	0,78
353	5,61	4,546	CDM = 0,92 g/L				0,202	g CDM/L/OD				

Appendix table 26: C245 Xall-medium B (run 1)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,97	0,076	0,00	0,00	27,90	2,72	3,20	2,82	3,26	0,00	0,00	0,00
2	5,95	0,774	0,00	0,00	28,24	2,19	3,32	2,92	3,33	0,00	0,05	0,00
17	5,80	2,308	1,61	4,03	23,08	0,00	3,20	2,87	0,00	0,00	0,67	0,00
41	5,72	2,621	2,04	9,54	17,42	0,00	2,28	2,81	0,00	0,00	0,91	0,00
65	5,73	2,545	2,08	13,00	14,35	0,00	1,23	2,57	0,00	0,00	1,06	0,19
137	5,77	3,021	2,17	19,94	6,00	0,00	0,00	2,31	0,00	0,00	1,48	0,43
185	5,57	3,160	2,16	22,08	2,94	0,00	0,00	2,73	0,00	0,00	1,76	0,57
233	5,67	3,876	1,99	22,88	1,14	0,00	0,00	2,66	0,00	0,00	2,10	0,72
305	5,63	4,611	1,86	22,35	0,00	0,00	0,00	2,09	0,00	0,00	2,53	1,04
353	5,53	5,958	1,70	21,57	0,00	0,00	0,00	2,03	0,00	0,00	2,76	1,19
353	2,91	5,958	CDM = 1,50 g/L				0,252	g CDM/L/OD				

Appendix table 27: C245 Xall-medium B (run 2)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,97	0,076	0,00	0,00	27,90	2,72	3,20	2,82	3,26	0,00	0,00	0,00
2	5,95	0,802	0,00	0,00	27,35	2,09	3,29	2,81	3,28	0,00	0,05	0,00
17	5,80	2,469	1,58	4,30	21,97	0,00	2,96	2,68	0,00	0,00	0,66	0,00
41	5,73	2,577	2,03	9,46	16,93	0,00	2,74	2,72	0,00	0,00	0,89	0,00
65	5,73	2,415	2,10	12,32	14,61	0,00	1,21	2,54	0,00	0,00	0,99	0,20
137	5,76	2,648	2,31	17,06	9,73	0,00	0,23	2,32	0,00	0,00	1,18	0,39
185	5,57	2,920	2,31	18,96	6,70	0,00	0,00	2,51	0,00	0,10	1,30	0,49
233	5,67	3,262	2,35	21,07	4,26	0,00	0,00	2,40	0,00	0,00	1,37	0,56
305	5,64	3,793	2,18	21,91	1,80	0,00	0,00	2,57	0,00	0,00	1,81	0,71
353	5,69	5,556	2,06	22,20	0,57	0,00	0,00	2,30	0,00	0,00	2,25	0,86
353	2,91	5,556	CDM = 1,22 g/L				0,220	g CDM/L/OD				

Appendix table 28: C255 Xall-medium A (run 1)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,13	0,073	0,00	0,00	29,05	2,90	4,30	3,48	4,35	0,00	0,00	0,00
1	6,12	0,744	0,00	0,00	28,43	2,78	4,10	3,42	4,21	0,00	0,00	0,00
18	5,93	3,243	0,94	0,00	26,11	1,16	5,65	3,37	2,47	0,00	0,34	0,00
42	5,76	5,118	1,96	0,27	25,38	0,16	4,88	3,52	0,93	0,00	0,76	0,00
66	5,88	5,118	2,36	1,22	24,18	0,00	4,19	3,45	0,55	0,00	0,87	0,00
138	5,82	5,442	3,29	3,88	22,35	0,00	0,00	3,18	0,36	0,00	1,01	0,12
186	5,80	5,928	3,82	5,17	19,09	0,00	0,00	3,13	0,29	0,00	1,02	0,29
234	5,55	7,460	4,19	6,04	15,50	0,00	0,00	2,76	0,00	0,00	1,09	0,55
306	5,19	11,864	4,52	6,46	9,63	0,00	0,00	2,55	0,00	0,00	1,10	0,79
354	4,64	13,400	4,86	6,92	6,09	0,00	0,00	2,55	0,00	0,00	1,13	0,93
402	3,84	14,212	5,37	12,59	3,70	0,00	0,00	3,60	0,00	0,00	1,14	1,74
497	2,92	23,600	4,93	5,49	0,00	0,00	0,00	1,21	0,00	0,00	0,48	0,63
497	2,91	23,600	CDM =6,86 g/L			0,291	g CDM/L/OD					

Appendix table 29: C255 Xall-medium A (run 2)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,13	0,073	0,00	0,00	29,05	2,90	4,30	3,48	4,35	0,00	0,00	0,00
1	6,12	0,731	0,00	0,00	28,60	2,77	4,07	3,40	4,25	0,00	0,00	0,00
18	5,93	3,103	0,90	0,00	26,97	1,25	5,40	3,44	2,66	0,00	0,32	0,00
42	5,93	5,256	2,03	0,38	25,78	0,00	4,57	3,64	0,82	0,00	0,80	0,00
66	5,87	6,128	2,42	1,84	24,23	0,00	4,20	3,63	0,57	0,00	1,06	0,00
138	5,76	6,130	3,52	5,59	19,78	0,00	0,00	3,20	0,37	0,00	1,29	0,21
186	5,71	7,190	3,87	6,22	15,83	0,00	0,00	3,05	0,26	0,00	1,38	0,41
234	5,45	7,954	4,30	6,76	12,39	0,00	0,00	2,70	0,00	0,00	1,41	0,68
306	5,15	11,548	5,06	7,69	6,93	0,00	0,00	2,60	0,00	0,00	1,45	0,92
354	4,87	12,456	5,61	8,53	3,57	0,00	0,00	2,65	0,00	0,00	1,46	1,09
402	4,13	13,952	6,21	14,73	0,44	0,00	0,00	3,09	0,00	0,00	1,56	2,02
497	3,21	18,480	5,24	7,67	0,00	0,00	0,00	0,99	0,00	0,00	1,15	0,86
497	3,21	18,480	CDM =4,96 g/L			0,268	g CDM/L/OD					

Appendix table 30: C255 Xall-medium B (run 1)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,13	0,079	0,00	0,00	28,39	2,84	4,07	3,38	4,29	0,00	0,00	0,00
1	6,12	0,754	0,00	0,00	28,66	2,74	3,97	3,44	4,23	0,00	0,00	0,00
18	5,94	3,715	1,31	0,00	26,47	0,84	5,01	3,43	2,11	0,00	0,46	0,00
42	5,98	5,222	2,35	1,46	25,21	0,00	3,94	3,62	0,61	0,00	0,86	0,00
66	5,90	5,234	2,82	3,60	21,77	0,00	3,29	3,33	0,44	0,00	0,91	0,00
138	5,86	5,218	3,46	6,96	18,67	0,00	0,00	3,04	0,34	0,00	0,90	0,32
186	5,83	6,774	3,92	8,02	14,76	0,00	0,00	3,01	0,27	0,00	0,94	0,58
234	5,42	9,208	4,22	8,67	10,36	0,00	0,00	2,69	0,00	0,00	0,95	0,87
306	4,76	15,596	4,66	9,13	3,93	0,00	0,00	2,65	0,00	0,00	0,94	1,13
354	4,13	17,220	4,80	9,44	0,35	0,00	0,00	2,43	0,00	0,00	0,83	1,35
402	3,70	18,824	4,84	12,11	0,00	0,00	0,00	1,51	0,00	0,00	0,58	1,84
497	3,35	25,700	4,20	6,47	0,00	0,00	0,00	0,00	0,00	0,00	0,22	1,26
497	3,36	25,700	CDM =7,54 g/L				0,293	g CDM/L/OD				

Appendix table 31: C255 Xall-medium B (run 2)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,13	0,079	0,00	0,00	28,39	2,84	4,07	3,38	4,29	0,00	0,00	0,00
1	6,13	0,753	0,26	0,00	28,14	2,71	3,88	3,33	4,06	0,00	0,07	0,00
18	5,95	3,556	1,21	0,00	26,14	0,93	4,62	3,32	2,20	0,00	0,43	0,00
42	5,93	5,220	2,23	1,11	24,93	0,00	3,92	3,52	0,66	0,00	0,87	0,00
66	5,90	5,428	2,67	3,31	21,51	0,00	3,44	3,32	0,46	0,00	0,98	0,00
138	5,84	5,462	3,45	6,94	18,02	0,00	0,00	2,98	0,35	0,00	1,05	0,29
186	5,83	6,490	3,69	7,58	15,59	0,00	0,00	3,01	0,26	0,00	1,08	0,46
234	5,66	6,882	3,85	8,35	14,14	0,00	0,00	2,74	0,00	0,13	1,04	0,65
306	5,53	9,848	4,20	8,64	10,97	0,00	0,00	2,61	0,00	0,00	1,03	0,79
354	5,38	12,164	4,35	8,90	8,11	0,00	0,00	2,49	0,00	0,00	1,00	0,90
402	5,18	12,832	4,93	14,39	6,97	0,00	0,23	3,36	0,00	0,12	1,09	1,50
497	4,62	14,912	5,08	9,87	0,40	0,00	0,00	1,90	0,00	0,00	0,91	1,15
497	4,58	14,912	CDM =4,62g/L				0,310	g CDM/L/OD				

Appendix table 32: HA1129 Xall-medium A (run 1)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,05	0,120	0,00	0,00	29,25	2,89	3,16	3,15	3,74	0,00	0,00	0,00
1,25	6,00	0,680	0,00	0,00	28,99	2,71	3,30	3,12	3,69	0,00	0,00	0,00
18,5	5,82	3,208	2,27	0,26	28,35	0,00	2,26	3,25	0,00	0,00	0,24	0,13
42,75	5,82	3,340	3,14	0,94	26,72	0,00	1,14	3,11	0,00	0,00	0,26	0,14
114,5	5,50	2,836	3,61	1,63	24,95	0,00	0,00	3,03	0,00	0,18	0,27	0,18
162,75	5,34	2,804	3,63	1,60	23,88	0,00	0,00	2,93	0,00	0,28	0,27	0,20
210,75	5,47	2,811	3,66	1,53	22,39	0,00	0,00	2,28	0,00	0,28	0,27	0,24
283,75	5,28	3,726	3,60	1,55	22,42	0,00	0,00	2,95	0,00	0,32	0,27	0,32
332,25	5,41	3,296	3,59	1,55	21,34	0,00	0,00	2,87	0,00	0,23	0,26	0,39
378,75	5,43	4,117	3,39	1,47	20,50	0,00	0,00	2,75	0,00	0,18	0,23	0,39
452	5,23	3,584	2,90	1,41	20,07	0,00	0,00	2,79	0,00	0,26	0,19	0,42
503,25	5,16	4,514	2,62	1,39	19,56	0,00	0,00	2,76	0,00	0,24	0,15	0,39
503,25	5,16	4,514	CDM = 2,28 g/L				0,505	g CDM/L/OD				

Appendix table 33: HA1129 Xall-medium A (run 2)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,05	0,120	0,00	0,00	29,25	2,89	3,16	3,15	3,74	0,00	0,00	0,00
1,25	6,06	0,692	0,00	0,00	29,04	2,69	3,10	3,13	3,63	0,00	0,00	0,00
18,5	5,86	2,857	2,25	0,29	28,60	0,00	2,36	3,28	0,00	0,00	0,24	0,11
42,75	5,84	3,203	3,20	0,85	26,35	0,00	0,85	3,02	0,00	0,00	0,29	0,15
114,5	5,43	3,063	3,41	1,26	24,97	0,00	0,00	3,03	0,00	0,20	0,29	0,24
162,75	5,29	2,939	3,26	1,16	23,23	0,00	0,00	2,92	0,00	0,24	0,29	0,26
210,75	5,33	2,992	3,21	1,40	21,49	0,00	0,00	2,27	0,00	0,29	0,29	0,33
283,75	5,28	3,825	2,91	1,52	20,49	0,00	0,00	2,77	0,00	0,22	0,23	0,43
332,25	5,31	4,186	2,65	1,58	19,40	0,00	0,00	2,63	0,00	0,14	0,19	0,45
378,75	5,29	4,119	2,20	1,60	19,04	0,00	0,00	2,88	0,00	0,11	0,14	0,52
452	4,92	4,985	1,36	1,53	17,20	0,00	0,00	2,63	0,00	0,00	0,05	0,60
503,25	4,87	6,074	1,10	1,58	16,73	0,00	0,00	2,61	0,00	0,00	0,12	0,64
503,25	4,87	6,074	CDM = 3,22 g/L				0,530	g CDM/L/OD				

Appendix table 34: HA1129 Xall-medium B (run 1)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,07	0,091	0,00	0,00	28,38	2,79	2,89	3,02	3,61	0,00	0,00	0,00
1,25	6,07	0,757	0,00	0,00	28,08	2,54	6,42	0,00	3,52	0,00	0,00	0,00
18,5	5,94	2,786	2,52	0,43	27,02	0,00	2,23	3,11	0,00	0,00	0,21	0,07
42,75	5,97	3,130	3,41	1,13	25,43	0,00	0,67	2,90	0,00	0,00	0,24	0,14
114,5	5,76	3,235	3,74	1,46	24,92	0,00	0,00	2,88	0,00	0,00	0,24	0,17
162,75	5,75	3,170	3,66	1,36	24,33	0,00	0,00	2,88	0,00	0,18	0,24	0,18
210,75	5,86	3,438	3,59	1,30	23,06	0,00	0,00	2,20	0,00	0,21	0,23	0,21
283,75	5,77	3,325	3,41	1,22	24,00	0,00	0,00	2,84	0,00	0,19	0,24	0,23
332,25	5,80	3,303	3,27	1,16	23,17	0,00	0,00	2,72	0,00	0,15	0,24	0,31
378,75	5,92	3,619	2,92	1,05	22,75	0,00	0,00	2,93	0,00	0,00	0,24	0,35
452	5,83	5,200	1,92	1,05	20,49	0,00	0,00	2,42	0,00	0,00	0,15	0,49
503,25	5,72	6,790	2,87	1,14	18,96	0,00	0,00	2,43	0,00	0,29	0,24	0,55
503,25	5,72	6,790	CDM = 3,30 g/L				0,486	g CDM/L/OD				

Appendix table 35: HA1129 Xall-medium B (run 2)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,07	0,091	0,00	0,00	28,38	2,79	2,89	3,02	3,61	0,00	0,00	0,00
1,25	6,06	0,747	0,00	0,00	28,08	2,54	3,10	2,99	3,57	0,00	0,00	0,00
18,5	5,93	3,036	2,53	0,45	26,89	0,00	2,22	3,05	0,00	0,00	0,21	0,09
42,75	5,97	3,467	3,46	1,17	25,96	0,00	0,69	2,99	0,00	0,00	0,24	0,13
114,5	5,76	4,128	3,69	1,51	24,51	0,00	0,00	2,89	0,00	0,18	0,24	0,16
162,75	5,70	3,531	3,66	1,51	24,02	0,00	0,00	2,87	0,00	0,27	0,25	0,21
210,75	5,78	3,887	3,68	1,50	23,53	0,00	0,00	2,84	0,00	0,29	0,24	0,21
283,75	5,64	3,801	3,48	1,43	23,14	0,00	0,00	2,85	0,00	0,35	0,24	0,23
332,25	5,66	3,192	3,41	1,37	22,65	0,00	0,00	2,81	0,00	0,35	0,24	0,25
378,75	5,70	3,217	3,30	1,31	22,52	0,00	0,00	2,81	0,00	0,35	0,24	0,28
452	5,65	4,005	3,03	1,18	21,74	0,00	0,00	2,74	0,00	0,33	0,24	0,36
503,25	5,65	4,338	1,01	1,08	21,38	0,00	0,00	2,82	0,00	0,00	0,00	0,39
503,25	5,65	4,338	CDM = 1,72 g/L				0,396	g CDM/L/OD				

9.7 Raw data of the cultivations in culture bottles with hydrolysate-medium

Appendix table 36: A027 hydrolysate-medium A (run 1)

Time [h]	pH	OD_{600}	OD - T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,67	5,350	0,000	0,00	0,00	28,44	13,06	4,02	1,45	0,04	0,00
0,5	5,62	6,852	1,502	0,15	0,00	28,80	12,50	4,18	1,31	0,10	0,00
17	5,29	7,684	2,334	3,59	0,00	29,39	0,00	3,62	0,20	1,11	0,00
113	5,20	9,024	3,674	4,06	0,08	27,62	0,00	3,72	0,42	1,30	0,07
161	4,91	9,204	3,854	3,86	0,00	26,44	0,00	3,39	0,58	1,42	0,00
209	4,87	9,608	4,258	3,58	0,00	26,60	0,00	3,63	0,60	1,44	0,13
329	4,86	11,100	5,750	2,77	0,00	25,26	0,00	3,53	0,63	1,53	0,15
377,25	4,84	11,368	6,018	2,56	0,16	26,52	0,00	4,11	0,63	1,60	0,17
449,25	4,82	12,120	6,770	2,06	0,15	24,70	0,00	4,02	0,58	1,62	0,19
473,5	4,77	12,256	6,906	1,81	0,13	24,16	0,00	4,07	0,55	1,63	0,20
473,5	4,77	6,906	CDM =3,14 g/L				0,455	g CDM/L/OD			

Appendix table 37: A027 hydrolysate-medium A (run 2)

Time [h]	pH	OD_{600}	OD - T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,67	5,350	0,000	0,00	0,00	28,44	13,06	4,02	1,45	0,04	0,00
0,5	5,64	6,620	1,270	0,22	0,00	29,17	12,60	4,19	1,41	0,11	0,00
17	5,29	7,580	2,230	3,51	0,00	28,60	0,00	3,59	0,23	1,08	0,00
113	5,12	9,540	4,190	3,90	0,11	27,33	0,00	3,70	0,60	1,28	0,10
161	4,80	9,804	4,454	3,70	0,00	25,72	0,00	3,23	0,81	1,33	0,00
209	4,78	9,872	4,522	3,43	0,00	26,50	0,00	3,72	0,92	1,35	0,13
329	4,71	10,780	5,430	2,76	0,00	25,10	0,00	3,68	1,15	1,39	0,18
377,25	4,68	10,816	5,466	2,60	0,14	25,48	0,00	3,88	1,19	1,45	0,21
449,25	4,66	11,084	5,734	2,19	0,15	23,65	0,00	3,82	1,15	1,46	0,20
473,5	4,64	11,068	5,718	2,09	0,16	22,72	0,00	4,00	1,17	1,47	0,20
473,5	4,64	5,718	CDM =2,62 g/L				0,458	g CDM/L/OD			

Appendix table 38: A027 hydrolysate-medium B (run 1)

Time [h]	pH	OD_{600}	OD - T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,73	5,784	0,000	0,00	0,00	29,15	13,64	4,16	1,26	0,04	0,00
0,5	5,73	7,352	1,568	0,00	0,00	29,54	12,96	4,25	1,23	0,09	0,00
17	5,37	8,072	2,288	3,57	0,00	29,16	0,00	3,64	0,19	1,18	0,00
113	5,18	9,660	3,876	4,29	0,12	27,82	0,00	3,69	0,34	1,37	0,09
161	5,03	9,804	4,020	4,19	0,00	26,92	0,00	3,40	0,52	1,44	0,00
209	4,93	9,868	4,084	4,03	0,00	27,21	0,00	3,67	0,71	1,46	0,11
329	4,81	10,836	5,052	3,44	0,00	25,64	0,00	3,67	0,98	1,55	0,19
377,25	4,78	10,992	5,208	3,41	0,20	24,79	0,00	3,44	1,06	1,62	0,23
449,25	4,74	11,432	5,648	3,20	0,20	23,50	0,00	3,54	1,05	1,62	0,22
473,5	4,67	11,512	5,728	3,20	0,21	23,32	0,00	3,89	1,11	1,66	0,24
473,5	4,67	5,728	CDM =2,88 g/L				0,503	g CDM/L/OD			

Appendix table 39: A027 hydrolysate-medium B (run 2)

Time [h]	pH	OD_{600}	OD - T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,73	5,784	0,000	0,00	0,00	29,15	13,64	4,16	1,26	0,04	0,00
0,5	5,67	7,108	1,324	0,00	0,00	29,20	13,05	4,14	1,22	0,09	0,00
17	5,37	8,270	2,486	3,60	0,00	30,07	0,00	3,75	0,19	1,16	0,00
113	5,28	9,576	3,792	4,27	0,11	27,70	0,00	3,43	0,39	1,36	0,10
161	4,99	10,000	4,216	4,22	0,00	26,37	0,00	3,32	0,60	1,46	0,00
209	4,91	10,124	4,340	4,00	0,00	26,57	0,00	3,59	0,78	1,45	0,14
329	4,79	11,192	5,408	3,42	0,00	25,04	0,00	3,59	1,06	1,49	0,17
377,25	4,75	11,224	5,440	3,46	0,13	24,29	0,00	3,38	1,18	1,57	0,22
449,25	4,68	11,756	5,972	3,22	0,18	23,97	0,00	3,59	1,18	1,58	0,22
473,5	4,64	12,124	6,340	3,17	0,16	23,32	0,00	3,98	1,26	1,62	0,21
473,5	4,64	6,340	CDM =3,02 g/L				0,476	g CDM/L/OD			

Appendix table 40: C238 hydrolysate-medium A (run 1)

Time [h]	pH	OD_{600}	OD - T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,67	5,350	0,000	0,00	0,00	28,44	13,06	4,02	1,45	0,04	0,00
0,5	5,59	6,452	1,102	0,12	0,00	28,37	13,00	4,09	1,40	0,06	0,00
17	5,43	8,560	3,210	3,60	0,22	28,78	0,00	3,95	1,36	0,63	0,00
113	5,35	17,960	12,610	3,96	1,27	23,03	0,00	3,49	1,18	0,50	0,00
161	5,06	23,785	18,435	4,01	1,46	19,49	0,00	3,23	1,02	0,38	0,00
209	4,95	27,420	22,070	4,08	1,85	16,76	0,00	3,18	1,08	0,25	0,00
329	4,83	34,620	29,270	4,17	2,33	9,60	0,00	2,91	0,89	0,12	0,23
377,25	4,80	35,710	30,360	4,18	2,50	7,30	0,00	2,63	0,66	0,09	0,29
449,25	4,76	38,940	33,590	3,14	2,62	2,72	0,00	2,72	0,58	0,05	0,36
473,5	4,72	41,280	35,930	2,15	2,63	0,00	0,00	3,02	0,07	0,05	0,43
473,5	4,72	35,930	CDM =11,54 g/L				0,321	g CDM/L/OD			

Appendix table 41: C238 hydrolysate-medium A (run 2)

Time [h]	pH	OD_{600}	OD - T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,67	5,350	0,000	0,00	0,00	28,44	13,06	4,02	1,45	0,04	0,00
0,5	5,60	6,412	1,062	0,10	0,00	28,76	12,68	4,14	1,25	0,05	0,00
17	5,42	8,184	2,834	3,69	0,19	28,42	0,00	3,73	1,43	0,64	0,00
113	5,37	12,760	7,410	4,24	0,44	25,65	0,00	3,30	1,32	0,67	0,06
161	5,15	14,375	9,025	4,57	0,60	24,39	0,00	3,42	1,33	0,70	0,00
209	5,17	16,505	11,155	4,93	0,83	22,24	0,00	3,16	1,32	0,72	0,11
329	5,07	22,580	17,230	5,96	1,09	16,10	0,00	2,79	1,29	0,77	0,18
377,25	5,03	24,670	19,320	6,36	1,21	14,37	0,00	3,06	1,31	0,75	0,19
449,25	4,98	26,460	21,110	6,72	1,26	10,40	0,00	2,21	1,28	0,66	0,21
473,5	4,89	27,090	21,740	7,00	1,27	9,20	0,00	2,74	1,28	0,55	0,23
473,5	4,89	21,740	CDM =7,48 g/L				0,344	g CDM/L/OD			

Appendix table 42: C238 hydrolysate-medium B (run 1)

Time [h]	pH	OD_{600}	OD - T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,73	5,784	0,000	0,00	0,00	29,15	13,64	4,16	1,26	0,04	0,00
0,5	5,68	7,016	1,232	0,15	0,00	28,63	13,04	4,21	1,20	0,05	0,00
17	5,51	9,172	3,388	3,63	0,16	29,23	0,00	3,82	1,20	0,66	0,00
113	5,43	14,276	8,492	4,59	0,97	23,86	0,00	3,64	1,13	0,63	0,05
161	5,19	17,710	11,926	4,93	1,42	20,92	0,00	3,30	0,58	1,05	0,00
209	5,19	20,110	14,326	5,16	1,64	18,74	0,00	3,08	0,90	0,51	0,00
329	5,11	27,190	21,406	5,77	1,95	12,76	0,00	2,91	0,09	0,46	0,11
377,25	5,06	29,590	23,806	6,10	2,10	10,14	0,00	2,65	0,12	0,45	0,18
449,25	5,00	30,920	25,136	5,97	2,28	6,62	0,00	2,09	0,13	0,33	0,25
473,5	4,99	33,680	27,896	5,58	2,35	6,50	0,00	3,02	0,10	0,10	0,32
473,5	4,99	27,896	CDM =9,14 g/L				0,328	g CDM/L/OD			

Appendix table 43: C238 hydrolysate-medium B (run 2)

Time [h]	pH	OD_{600}	OD - T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,73	5,784	0,000	0,00	0,00	29,15	13,64	4,16	1,26	0,04	0,00
0,5	5,67	6,896	1,112	0,00	0,00	28,76	13,09	4,10	1,21	0,05	0,00
17	5,52	8,726	2,942	3,71	0,18	29,52	0,00	3,96	1,20	0,65	0,00
113	5,50	11,588	5,804	4,12	0,38	27,86	0,00	3,60	1,17	0,63	0,07
161	5,26	12,910	7,126	4,43	0,45	26,74	0,00	3,50	0,64	1,14	0,00
209	5,26	14,135	8,351	4,70	0,58	26,05	0,00	3,40	1,33	0,63	0,10
329	5,19	15,135	9,351	5,20	0,78	22,15	0,00	3,20	1,04	0,66	0,13
377,25	5,18	18,025	12,241	5,77	0,82	19,65	0,00	3,02	1,04	0,68	0,15
449,25	5,10	24,085	18,301	5,69	0,97	14,42	0,00	2,44	0,95	0,50	0,12
473,5	5,00	29,270	23,486	5,55	1,29	13,22	0,00	3,46	0,90	0,38	0,16
473,5	5,00	23,486	CDM =8,30 g/L				0,353	g CDM/L/OD			

Appendix table 44: C245 hydrolysate-medium A (run 1)

Time [h]	pH	OD_{600}	OD - T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,67	5,350	0,000	0,00	0,00	28,44	13,06	4,02	1,45	0,04	0,00
0,5	5,69	6,440	1,090	0,00	0,00	28,45	12,93	4,06	1,27	0,06	0,00
17	5,36	6,132	0,782	1,57	0,23	28,18	8,59	3,66	1,40	0,86	0,13
113	5,28	8,216	2,866	2,97	5,47	21,55	0,00	3,59	1,19	2,09	0,31
161	5,10	8,768	3,418	2,93	7,83	18,23	0,00	3,38	1,14	2,31	0,33
209	5,11	9,484	4,134	2,78	10,19	15,25	0,00	3,68	1,30	2,68	0,47
329	5,03	12,115	6,765	2,04	12,70	9,52	0,00	3,47	1,14	3,70	0,51
377,25	4,99	13,015	7,665	1,84	13,58	8,04	0,00	3,30	1,11	4,01	0,45
449,25	4,91	14,895	9,545	1,29	13,68	6,95	0,00	3,45	0,74	4,12	0,39
473,5	4,84	15,640	10,290	0,89	13,65	6,08	0,00	3,37	0,70	3,85	0,36
473,5	4,84	10,290	CDM =4,48 g/L				0,435	g CDM/L/OD			

Appendix table 45: C245 hydrolysate-medium A (run 2)

Time [h]	pH	OD_{600}	OD - T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,67	5,350	0,000	0,00	0,00	28,44	13,06	4,02	1,45	0,04	0,00
0,5	5,60	6,548	1,198	0,11	0,00	28,66	13,01	4,10	1,45	0,08	0,00
17	5,37	6,126	0,776	1,58	0,21	28,37	8,96	3,71	1,43	0,86	0,11
113	5,30	13,880	8,530	2,61	7,84	17,09	0,00	3,47	1,32	3,05	0,42
161	5,11	9,945	4,595	2,53	9,78	13,99	0,00	3,29	1,34	3,54	0,42
209	5,09	10,624	5,274	2,39	10,89	12,76	0,00	3,71	1,25	3,84	0,58
329	5,07	11,975	6,625	1,79	11,65	8,59	0,00	3,45	1,05	4,16	0,49
377,25	5,00	12,770	7,420	1,74	13,09	7,93	0,00	3,40	1,09	4,53	0,45
449,25	4,94	13,880	8,530	1,36	12,85	7,51	0,00	3,87	0,72	4,52	0,36
473,5	4,88	21,824	16,474	1,05	12,86	6,39	0,00	3,57	0,68	4,37	0,36
473,5	4,88	16,474	CDM =4,46 g/L				0,271	g CDM/L/OD			

Appendix table 46: C245 hydrolysate-medium B (run 1)

Time [h]	pH	OD_{600}	OD - T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,73	5,784	0,000	0,00	0,00	29,15	13,64	4,16	1,26	0,04	0,00
0,5	5,66	7,128	1,344	0,00	0,00	29,19	12,86	4,21	1,22	0,07	0,00
17	5,47	6,502	0,718	1,48	0,18	28,58	9,08	3,69	1,20	0,81	0,14
113	5,36	15,864	10,080	3,28	3,96	20,10	0,00	3,34	1,16	1,84	0,29
161	5,11	20,685	14,901	3,22	4,18	17,47	0,00	3,27	1,05	1,80	0,19
209	5,09	24,180	18,396	3,12	4,66	15,71	0,00	3,49	0,91	1,62	0,23
329	4,97	34,620	28,836	2,68	5,38	9,78	0,00	3,36	0,69	1,05	0,28
377,25	4,90	33,870	28,086	2,36	5,88	7,67	0,00	2,92	0,63	0,75	0,32
449,25	4,84	36,300	30,516	1,34	6,04	6,85	0,00	3,49	0,52	0,56	0,41
473,5	4,80	37,650	31,866	0,50	5,85	5,10	0,00	3,14	0,46	0,10	0,43
473,5	4,80	31,866	CDM =12 g/L				0,377	g CDM/L/OD			

Appendix table 47: C245 hydrolysate-medium B (run 2)

Time [h]	pH	OD_{600}	OD - T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,73	5,784	0,000	0,00	0,00	29,15	13,64	4,16	1,26	0,04	0,00
0,5	5,67	6,880	1,096	0,00	0,00	29,42	13,23	4,20	1,21	0,07	0,00
17	5,47	6,386	0,602	1,45	0,17	28,99	9,22	3,76	1,20	0,85	0,14
113	5,33	12,108	6,324	3,25	4,42	20,89	0,00	3,45	1,13	2,35	0,44
161	5,16	15,495	9,711	3,48	5,10	17,93	0,00	3,25	1,11	2,19	0,33
209	5,15	18,380	12,596	3,53	5,81	16,67	0,00	3,48	0,99	2,43	0,41
329	5,06	30,040	24,256	3,40	6,19	11,53	0,00	3,24	0,84	1,93	0,26
377,25	5,03	31,600	25,816	3,47	6,66	9,72	0,00	2,98	0,82	1,69	0,24
449,25	4,97	35,680	29,896	2,87	6,66	7,68	0,00	3,01	0,71	0,77	0,31
473,5	4,92	36,850	31,066	2,45	6,65	6,68	0,00	3,19	0,69	0,43	0,36
473,5	4,92	31,066	CDM =9,58 g/L				0,308	g CDM/L/OD			

Appendix table 48: C255 hydrolysate-medium A (run 1)

Time [h]	pH	OD_{600}	OD - T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,94	1,108	1,108	0,00	0,00	24,25	6,22	3,49	0,44	0,00	0,00
0,5	5,92	1,694	0,586	0,00	0,00	24,74	6,16	3,53	0,42	0,03	0,00
17,5	5,91	3,545	2,437	0,90	0,00	22,97	2,49	3,02	0,40	0,71	0,07
41,75	5,84	3,645	2,537	1,42	1,16	21,04	0,00	3,07	0,39	1,01	0,15
65,5	5,92	4,077	2,969	1,51	1,97	19,43	0,00	3,07	0,39	1,03	0,15
138,5	5,76	6,558	5,450	1,81	4,80	14,11	0,00	3,02	0,35	1,20	0,51
187	5,77	7,958	6,850	2,04	6,95	9,82	0,00	2,83	0,32	1,26	0,84
234,5	5,79	11,392	10,284	2,01	7,90	6,48	0,00	2,64	0,24	1,11	1,03
306,75	5,71	13,836	12,728	2,22	7,95	0,00	0,00	2,14	0,06	1,04	1,13
354,75	5,67	16,308	15,200	1,96	7,56	0,00	0,00	1,89	0,00	0,82	0,82
401,75	5,50	18,012	16,904	1,74	7,32	0,00	0,00	1,52	0,00	0,59	0,77
475	5,65	25,896	24,788	1,13	6,49	0,00	0,00	1,07	0,00	0,15	0,78
475	5,65	24,788	CDM =7,62 g/L				0,307 g CDM/L/OD				

Appendix table 49: C255 hydrolysate-medium A (run 2)

Time [h]	pH	OD_{600}	OD - T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,94	1,108	1,108	0,00	0,00	24,25	6,22	3,49	0,44	0,00	0,00
0,5	5,91	1,808	0,700	0,00	0,00	23,73	5,88	3,26	0,44	0,03	0,00
17,5	5,91	3,358	2,250	0,87	0,00	23,04	2,05	3,09	0,42	0,65	0,00
41,75	5,84	3,521	2,413	1,43	1,01	21,71	0,00	3,09	0,41	1,00	0,13
65,5	5,93	3,627	2,519	1,56	1,73	19,93	0,00	2,92	0,40	1,04	0,16
138,5	5,74	7,062	5,954	1,81	3,27	15,62	0,00	2,89	0,37	1,20	0,36
187	5,72	8,732	7,624	2,08	4,37	12,31	0,00	2,85	0,33	1,32	0,63
234,5	5,72	11,632	10,524	2,32	5,37	9,15	0,00	2,80	0,30	1,38	0,89
306,75	5,58	15,464	14,356	2,60	6,00	5,32	0,00	2,52	0,24	1,26	1,00
354,75	5,44	18,864	17,756	2,68	6,04	0,00	0,00	2,25	0,08	1,05	0,76
401,75	5,23	21,752	20,644	2,65	5,78	0,00	0,00	1,86	0,00	0,45	0,72
475	5,41	32,752	31,644	2,33	4,42	0,00	0,00	1,20	0,00	0,00	0,56
475	5,41	31,644	CDM =8,7 g/L				0,275 g CDM/L/OD				

Appendix table 50: C255 hydrolysate-medium B (run 1)

Time [h]	pH	OD_{600}	OD - T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,93	1,179	1,179	0,00	0,00	23,80	6,16	3,19	0,43	0,00	0,00
0,5	5,93	1,741	0,562	0,00	0,00	23,75	5,96	3,30	0,41	0,00	0,00
17,5	5,92	3,568	2,389	0,97	0,00	23,17	2,54	3,13	0,39	0,76	0,05
41,75	5,86	4,319	3,140	1,41	1,39	19,90	0,00	2,90	0,37	1,05	0,14
65,5	5,92	4,467	3,288	1,52	2,60	18,77	0,00	2,95	0,38	1,12	0,20
138,5	5,78	8,592	7,413	1,66	4,09	15,07	0,00	3,08	0,35	1,19	0,46
187	5,74	11,108	9,929	1,74	4,01	12,57	0,00	2,86	0,30	1,16	0,59
234,5	5,72	13,920	12,741	1,66	4,19	10,29	0,00	2,78	0,26	1,09	0,74
306,75	5,60	16,552	15,373	1,84	4,71	6,91	0,00	2,61	0,11	1,15	0,93
354,75	5,55	19,140	17,961	1,84	4,70	5,13	0,00	2,42	0,07	1,13	1,01
401,75	5,41	19,896	18,717	1,33	4,74	0,00	0,00	2,15	0,00	0,88	0,95
475	5,51	27,760	26,581	0,93	4,40	0,00	0,00	1,73	0,00	0,51	0,83
475	5,51	26,581	CDM =8,1 g/L				0,305	g CDM/L/OD			

Appendix table 51: C255 hydrolysate-medium B (run 2)

Time [h]	pH	OD_{600}	OD - T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,93	1,179	1,179	0,00	0,00	23,80	6,16	3,19	0,43	0,00	0,00
0,5	5,94	1,712	0,533	0,00	0,00	23,80	5,92	3,31	0,41	0,03	0,00
17,5	5,92	3,352	2,173	0,89	0,00	23,01	2,53	3,05	0,39	0,68	0,00
41,75	5,84	3,741	2,562	1,39	1,18	21,17	0,00	3,02	0,37	1,04	0,16
65,5	5,93	3,865	2,686	1,53	1,90	19,79	0,00	2,98	0,38	1,08	0,14
138,5	5,80	6,248	5,069	1,83	3,39	16,17	0,00	2,99	0,34	1,14	0,36
187	5,78	9,556	8,377	1,84	3,33	14,07	0,00	2,92	0,34	1,12	0,48
234,5	5,75	13,128	11,949	1,95	3,35	11,77	0,00	2,88	0,31	1,26	0,66
306,75	5,60	16,232	15,053	2,00	3,78	8,14	0,00	2,66	0,23	1,21	0,84
354,75	5,53	19,160	17,981	2,09	3,96	5,78	0,00	2,50	0,19	1,20	0,94
401,75	5,34	22,476	21,297	1,91	3,94	0,00	0,00	2,17	0,16	0,92	0,77
475	5,33	33,600	32,421	1,46	3,40	0,00	0,00	1,76	0,00	0,21	0,63
475	5,33	32,421	CDM =9,3 g/L				0,287	g CDM/L/OD			

9.8 Raw data of the fermenter runs with Xall-medium

Appendix table 52: A027 Xall-medium B (run 1)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,07	0,139	0,00	0,00	28,81	2,82	2,77	2,93	3,70	0,00	0,00	0,00
2	6,07	0,890	0,00	0,00	28,49	2,33	2,77	2,91	3,60	0,00	0,00	0,00
11	5,74	8,640	3,98	0,69	27,61	0,00	0,00	2,78	0,00	0,23	0,48	0,17
33,5	5,35	10,560	4,62	1,39	23,27	0,00	0,00	2,26	0,00	0,36	0,51	0,49
105,75	5,01	14,050	6,93	2,06	15,25	0,00	0,00	1,39	0,00	0,45	0,60	1,05
129,5	4,89	14,520	7,71	2,33	13,61	0,00	0,00	1,72	0,00	0,46	0,64	1,26
153,5	4,85	15,020	8,31	2,33	11,10	0,00	0,00	1,54	0,00	0,45	0,67	1,28
177,5	4,63	14,985	8,76	2,25	9,50	0,00	0,00	1,44	0,00	0,42	0,67	1,35
203	4,71	15,215	9,33	2,35	8,68	0,00	0,00	1,42	0,00	0,43	0,71	1,37
273,5	4,59	14,895	9,72	2,25	5,17	0,00	0,00	1,11	0,00	0,43	0,72	1,41
297,5	4,44	15,320	9,76	2,30	4,42	0,00	0,00	1,15	0,00	0,39	0,73	1,53
321,5	4,41	15,205	9,80	2,31	3,65	0,00	0,00	1,19	0,00	0,36	0,73	1,55
324,75	4,39	15,045	9,92	2,30	4,00	0,00	0,00	1,56	0,00	0,35	0,75	1,55
325	4,37	12,108	8,29	1,87	100,02	0,24	0,00	11,45	0,00	0,30	0,61	1,28
345,5	4,36	13,805	8,88	1,82	98,24	0,00	0,00	11,51	0,00	0,25	0,58	1,25
369,5	4,20	13,780	9,40	1,80	95,28	0,00	0,00	11,23	0,00	0,33	0,71	1,27
489,5	3,86	13,765	10,73	1,89	88,92	0,00	0,00	10,79	0,00	0,84	0,83	1,54
513,75	3,98	13,515	10,87	1,93	90,32	0,00	0,00	11,10	0,00	1,14	0,86	1,56
537,5	3,75	12,860	10,29	1,89	89,63	0,00	0,00	11,10	0,00	1,39	0,86	1,56
609,5	3,61	12,075	9,56	1,88	88,07	0,00	0,00	10,95	0,00	2,19	0,85	1,58
633,5	3,60	12,610	9,24	1,86	87,41	0,00	0,00	10,84	0,00	2,46	0,85	1,59
633,5	3,60	12,610	CDM = 2,34 g/L			0,186	g CDM/L/OD					
			CDM = 2,26 g/L			0,179	g CDM/L/OD	**mean:**	**0,188**	**g CDM/L/OD**		
			CDM = 2,50 g/L			0,198	g CDM/L/OD					

Appendix table 53: A027 Xall-medium B (run 2)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,08	0,109	0,00	0,00	28,08	2,73	2,91	2,89	3,61	0,00	0,00	0,00
1,75	6,04	0,826	0,00	0,00	28,03	2,35	2,99	2,90	3,60	0,00	0,00	0,00
17,5	5,66	7,784	3,77	0,43	26,96	0,00	0,00	2,85	0,00	0,22	0,44	0,11
41,5	5,33	9,042	4,49	1,06	24,11	0,00	0,00	2,51	0,00	0,38	0,47	0,48
67	5,22	12,768	5,50	1,34	22,59	0,00	0,00	2,38	0,00	0,45	0,51	0,77
137,5	5,01	13,880	6,59	1,29	14,91	0,00	0,00	1,71	0,00	0,41	0,52	0,99
161,5	4,81	14,424	7,01	1,33	13,59	0,00	0,00	1,68	0,00	0,38	0,53	1,07
185,5	4,77	14,764	7,19	1,35	11,91	0,00	0,00	1,67	0,00	0,32	0,54	1,16
209,5	4,92	15,136	7,46	1,38	10,02	0,00	0,00	1,52	0,00	0,23	0,41	1,21
233,5	4,71	15,048	8,04	1,51	7,97	0,00	0,00	1,35	0,00	0,24	0,60	1,15
353,5	4,61	14,455	9,99	2,32	2,91	0,00	0,00	1,68	0,00	0,16	0,72	1,38
358,5	4,63	14,275	10,30	2,20	2,90	0,00	0,00	1,81	0,00	0,15	0,74	1,39
358,75	4,56	11,688	8,49	1,80	96,32	0,24	0,00	11,05	0,00	0,08	0,61	1,13
377,75	4,67	13,230	9,25	1,82	94,97	0,00	0,00	11,07	0,00	0,14	0,67	1,17
401,75	4,33	13,580	10,01	1,92	95,45	0,00	0,00	11,27	0,00	0,18	0,76	1,30
473,5	4,08	13,560	11,16	1,91	87,80	0,00	0,00	10,70	0,00	0,27	0,92	1,48
498	4,11	14,275	11,61	1,94	87,89	0,00	0,00	10,83	0,00	0,30	0,96	1,55
521,5	4,06	13,420	11,95	1,97	86,76	0,00	0,00	10,87	0,00	0,34	1,00	1,56
546	4,03	14,105	11,95	1,90	83,17	0,00	0,00	10,52	0,00	0,36	1,00	1,53
546	4,03	14,105	CDM = 2,66 g/L			0,189	g CDM/L/OD					
			CDM = 2,76 g/L			0,196	g CDM/L/OD	**mean:**	**0,192**	**g CDM/L/OD**		
			CDM = 2,72 g/L			0,193	g CDM/L/OD					

Appendix table 54: C238 Xall-medium B (run 1)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,04	0,116	0,00	0,00	29,24	2,87	3,84	3,44	4,34	0,00	0,00	0,00
0,75	6,01	0,545	0,00	0,00	29,29	2,72	3,72	3,47	4,23	0,00	0,00	0,00
17	5,97	5,177	3,18	2,10	25,16	0,00	1,17	3,54	0,00	0,00	0,34	0,00
24	5,95	6,138	3,56	3,22	24,28	0,00	1,34	3,53	0,00	0,00	0,37	0,00
41	5,81	6,390	3,62	4,14	22,79	0,00	0,00	3,33	0,00	0,13	0,38	0,00
47,5	5,79	6,330	3,65	4,51	22,28	0,00	0,00	3,23	0,00	0,17	0,40	0,00
113	5,49	8,136	3,64	9,90	12,86	0,00	0,00	2,95	0,00	0,16	0,40	0,30
120	5,49	8,080	3,54	10,41	11,64	0,00	0,00	2,95	0,00	0,29	0,41	0,32
137	5,52	8,252	3,56	12,85	9,15	0,00	0,00	3,04	0,00	0,28	0,43	0,41
161	5,48	8,580	3,77	15,11	3,03	0,00	0,00	1,98	0,00	0,21	0,47	0,53
185	5,31	10,500	4,07	15,76	1,00	0,00	0,00	2,17	0,00	0,17	0,47	0,67
193,25	5,29	10,132	4,11	15,38	0,24	0,00	0,00	2,00	0,00	0,00	0,46	0,75
193,75	5,15	7,424	3,55	13,02	92,86	0,00	0,00	11,20	0,00	0,00	0,38	0,65
209	4,74	6,130	4,33	15,17	86,23	0,00	0,00	10,97	0,00	0,00	0,64	0,94
233	3,42	21,345	7,26	28,30	58,84	0,00	0,00	9,92	0,00	0,00	1,10	1,82
257	3,35	21,515	8,34	33,46	46,74	0,00	0,00	9,39	0,00	0,00	1,20	2,03
281	3,18	23,760	9,70	38,33	34,61	0,00	0,00	8,75	0,00	0,00	1,33	2,29
305	3,22	22,155	9,91	39,67	32,87	0,00	0,00	8,86	0,00	0,00	1,33	2,37
329	3,12	21,985	10,93	44,15	25,15	0,00	0,00	8,10	0,00	0,00	1,52	2,87
401,25	2,68	26,605	14,87	49,89	2,71	0,00	0,00	7,82	0,00	0,00	1,93	3,88
425	2,68	34,755	15,49	49,83	1,19	0,00	0,00	7,56	0,00	0,00	1,74	4,34
449	2,71	30,950	15,70	49,96	0,71	0,00	0,00	7,35	0,00	0,00	1,75	4,61
473	2,75	31,900	15,98	48,50	0,45	0,00	0,00	7,28	0,00	0,00	1,76	4,66
473	2,75	31,900	CDM = 8,622 g/L			0,270	g CDM/L/OD					
			CDM = 8,18 g/L			0,256	g CDM/L/OD	**mean:**	**0,271**	**g CDM/L/OD**		
			CDM = 9,10 g/L			0,285	g CDM/L/OD					

Appendix table 55: C238 Xall-medium B (run 2)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,01	0,091	0,00	0,00	28,45	2,78	3,92	3,09	3,48	0,00	0,00	0,00
1	5,97	0,539	0,00	0,00	27,80	2,53	3,69	3,03	3,43	0,00	0,00	0,00
19	5,82	5,396	3,65	2,63	24,04	0,00	0,00	2,84	0,00	0,00	0,40	0,00
25	5,81	6,320	3,77	3,50	24,58	0,00	0,00	3,08	0,00	0,00	0,41	0,00
43	5,71	6,766	3,84	4,47	20,15	0,00	0,00	2,61	0,00	0,00	0,40	0,00
47,5	5,71	6,624	3,75	5,00	20,24	0,00	0,00	2,61	0,00	0,18	0,41	0,00
115	5,54	9,204	3,87	13,03	6,97	0,00	0,00	2,03	0,00	0,28	0,47	0,46
139	5,43	9,500	4,14	15,85	2,09	0,00	0,00	1,91	0,00	0,24	0,45	0,65
148	5,37	10,296	4,26	15,86	0,81	0,00	0,00	2,07	0,00	0,19	0,43	0,79
148,25	5,28	8,518	3,63	13,16	87,50	0,00	0,00	10,48	0,00	0,00	0,36	0,65
163	5,09	12,496	4,35	16,60	85,45	0,00	0,00	10,80	0,00	0,00	0,57	0,90
187	4,78	14,030	5,13	19,84	78,39	0,00	0,00	10,64	0,00	0,00	0,67	1,07
211	4,44	15,825	5,90	23,56	67,99	0,00	0,00	10,25	0,00	0,00	0,80	1,24
283	4,03	19,960	7,89	32,46	47,91	0,00	0,00	9,64	0,00	0,00	1,03	1,84
307	3,89	17,855	8,16	33,91	49,42	0,00	0,00	10,01	0,00	0,00	1,06	1,92
331	3,87	17,745	8,36	33,82	44,16	0,00	0,00	9,50	0,00	0,00	1,10	1,98
355	4,04	18,280	8,58	37,23	39,83	0,00	0,00	9,45	0,00	0,00	1,21	2,12
379	3,94	18,740	8,90	41,13	36,24	0,00	0,00	9,58	0,00	0,00	1,39	2,31
451	3,98	17,920	9,29	49,21	22,63	0,00	0,00	8,99	0,00	0,00	1,66	2,59
475	3,88	16,735	9,51	52,06	18,73	0,00	0,00	8,94	0,00	0,00	1,78	2,72
475	3,88	16,735	CDM = 4,96 g/L			0,296	g CDM/L/OD					
			CDM = 5,06 g/L			0,302	g CDM/L/OD	**mean:**	**0,303**	**g CDM/L/OD**		
			CDM = 5,20 g/L			0,311	g CDM/L/OD					

Appendix table 56: C245 Xall-medium B (run 1)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,07	0,114	0,00	0,00	30,33	3,04	3,49	3,08	3,44	0,00	0,00	0,00
2	6,04	0,574	0,00	0,00	29,87	2,61	3,31	3,02	3,36	0,00	0,00	0,00
21,25	5,80	1,955	1,34	4,44	24,09	0,00	2,82	2,75	1,71	0,00	0,69	0,00
25,75	5,79	2,030	1,34	6,01	22,09	0,00	2,91	2,87	1,37	0,00	0,83	0,00
45,25	5,73	2,552	1,35	13,28	13,34	0,00	1,99	2,69	0,57	0,00	1,81	0,11
51,75	5,73	2,836	1,33	15,15	12,10	0,00	2,10	2,80	0,45	0,00	2,08	0,14
117,5	5,49	4,232	1,16	20,17	1,18	0,00	0,00	3,09	0,00	0,00	5,57	0,37
123,25	5,51	4,232	1,18	20,77	0,66	0,00	0,00	2,74	0,00	0,00	5,91	0,41
123,75	5,41	4,395	0,98	17,15	90,50	0,26	0,00	11,31	0,00	0,00	5,03	0,34
141,25	5,37	4,750	0,96	23,02	83,13	0,00	0,00	11,28	0,00	0,00	5,70	0,49
165,25	5,41	4,900	0,92	32,03	75,27	0,00	0,00	11,49	0,00	0,00	7,00	0,70
189,25	5,35	4,808	0,85	37,64	63,53	0,00	0,00	10,78	0,00	0,00	8,08	0,83
213,25	5,30	5,206	0,78	43,79	54,50	0,00	0,00	10,58	0,00	0,00	9,18	1,00
308,25	5,09	5,380	0,59	67,03	19,61	0,00	0,00	9,60	0,00	0,00	14,69	2,04
332,25	5,05	5,426	0,52	71,37	13,31	0,00	0,00	9,25	0,00	0,00	16,10	2,44
356,25	4,95	5,840	0,50	73,01	7,45	0,00	0,00	7,69	0,00	0,00	17,53	2,91
380,25	4,92	6,524	0,45	73,73	4,33	0,00	0,00	7,55	0,00	0,00	19,03	3,50
452,25	4,63	6,922	0,43	71,08	0,24	0,00	0,00	5,48	0,00	0,00	23,00	6,28
456,75	4,63	7,660	0,43	70,36	0,00	0,00	0,00	5,34	0,00	0,00	23,38	6,47
456,75	4,58	7,660	CDM = 2,46 g/L			0,321	g CDM/L/OD					
			CDM = 2,46 g/L			0,321	g CDM/L/OD	**mean:**	**0,315**	**g CDM/L/OD**		
			CDM = 2,40 g/L			0,313	g CDM/L/OD					
			CDM = 2,32 g/L			0,303	g CDM/L/OD					

Appendix table 57: C245 Xall-medium B (run 2)

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,98	0,127	0,00	0,00	28,62	2,74	3,76	2,90	3,54	0,00	0,00	0,00
2	5,96	0,771	0,00	0,00	28,29	2,33	3,65	2,88	3,53	0,00	0,00	0,00
11	5,83	2,218	1,42	4,17	24,28	0,00	2,93	3,04	1,94	0,00	0,71	0,00
26	5,80	2,395	1,48	6,54	21,88	0,00	2,77	3,03	1,42	0,00	0,87	0,00
35	5,75	2,943	1,53	13,09	13,13	0,00	1,76	2,71	0,51	0,00	1,62	0,11
48	5,77	2,954	1,52	14,44	11,81	0,00	1,61	2,71	0,45	0,00	1,83	0,12
107	5,67	4,613	1,52	20,94	1,02	0,00	0,00	3,00	0,00	0,00	5,42	0,34
114	5,70	4,777	1,47	20,00	0,63	0,00	0,00	3,31	0,00	0,00	5,52	0,38
114,25	5,60	4,539	1,21	16,91	87,59	0,00	0,00	11,29	0,00	0,00	4,80	0,32
131	5,47	5,154	1,22	22,46	83,26	0,00	0,00	11,40	0,00	0,00	5,42	0,47
155	5,46	5,144	1,18	29,51	75,60	0,00	0,00	11,44	0,00	0,00	6,55	0,67
179	5,47	5,112	1,16	35,60	64,26	0,00	0,00	11,05	0,00	0,00	7,83	0,82
203	5,42	5,030	1,10	41,33	58,99	0,00	0,00	11,15	0,00	0,00	8,64	1,02
275	5,37	5,538	0,91	55,05	36,43	0,00	0,00	10,24	0,00	0,00	11,74	1,55
299	5,28	5,572	0,96	57,23	33,66	0,00	0,00	10,13	0,00	0,00	12,64	1,77
323	5,36	6,440	0,90	61,45	28,32	0,00	0,00	9,95	0,00	0,00	13,61	2,08
348,5	5,33	5,560	0,85	65,18	24,76	0,00	0,00	9,87	0,00	0,00	14,32	2,36
371	5,11	5,428	0,77	64,97	20,03	0,00	0,00	9,28	0,00	0,00	15,05	2,54
443,25	5,05	5,188	1,07	65,04	16,79	0,00	0,00	8,74	0,00	0,00	15,53	3,04
447,5	5,02	7,212	1,09	66,75	17,39	0,00	0,00	8,94	0,00	0,00	15,31	3,15
447,5	5,02	7,212	CDM = 1,88 g/L			0,261	g CDM/L/OD					
			CDM = 1,70 g/L			0,236	g CDM/L/OD	**mean:**	**0,285**	**g CDM/L/OD**		
			CDM = 2,58 g/L			0,358	g CDM/L/OD					

Appendix table 58: C245 Xall-medium A

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	6,11	0,108	0,00	0,00	28,35	2,81	3,25	2,97	3,71	0,00	0,00	0,00
1,75	6,03	0,675	0,00	0,00	28,11	2,53	3,08	2,93	3,66	0,00	0,00	0,00
17,5	5,81	1,763	0,98	2,40	25,38	0,18	2,91	2,92	3,34	0,00	0,59	0,00
41,5	5,66	2,595	1,00	9,90	16,34	0,00	2,09	2,79	1,15	0,00	1,66	0,00
67	5,66	3,395	0,79	15,39	9,19	0,00	2,09	2,92	0,54	0,00	3,24	0,20
137,5	5,33	5,224	0,90	18,22	0,00	0,00	0,00	2,34	0,00	0,00	6,53	0,90
142,75	5,29	5,390	0,84	17,87	0,00	0,00	0,00	1,64	0,00	0,00	6,65	1,13
143	5,15	4,723	0,71	14,55	91,50	0,18	0,00	11,62	0,00	0,00	5,60	0,94
161,5	4,94	6,178	0,94	20,34	87,05	0,00	0,00	11,82	0,00	0,00	6,28	1,24
185,5	4,82	6,250	0,97	28,71	73,39	0,00	0,00	10,62	0,00	0,00	7,18	1,51
209,5	4,77	5,684	0,79	37,95	61,24	0,00	0,00	10,46	0,00	0,00	8,28	1,74
233,5	4,54	6,062	0,99	47,86	48,69	0,00	0,00	10,57	0,00	0,00	9,31	2,15
353,5	3,82	6,714	0,96	78,14	2,06	0,00	0,00	7,66	0,00	0,00	15,05	5,10
377,75	3,76	6,398	0,86	76,23	0,94	0,00	0,00	7,10	0,00	0,00	16,55	6,10
401,5	3,32	6,844	0,67	75,26	0,31	0,00	0,00	6,57	0,00	0,00	17,57	6,86
473,5	2,90	6,246	0,00	73,46	0,00	0,00	0,00	6,11	0,00	0,00	17,59	7,12
476,5	2,86	6,758	0,00	74,18	0,00	0,00	0,00	6,10	0,00	0,00	17,70	7,12
476,5	2,86	17,655	0,00	72,42	0,00	0,00	0,00	6,29	0,00	0,00	17,24	7,01

without biomass film			CDM = 1,38 g/L	0,204	g CDM/L/OD		
476,5	2,86	6,758	CDM = 1,50 g/L	0,222	g CDM/L/OD	**mean: 0,212**	**g CDM/L/OD**
			CDM = 1,42 g/L	0,210	g CDM/L/OD		
with biomass film			CDM = 5,78 g/L	0,327	g CDM/L/OD		
476,5	2,86	0,306	CDM = 5,40 g/L	0,222	g CDM/L/OD	**mean: 0,316**	**g CDM/L/OD**
		0,314	CDM = 5,54 g/L	0,210	g CDM/L/OD		

Appendix table 59: C245 Xall-medium A with 3-fold inoculum

Time [h]	pH	OD_{600}	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Galactose [g/L]	Arabinose [g/L]	Mannose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,97	0,092	0,00	0,00	29,24	2,86	3,30	3,12	3,64	0,00	0,00	0,00
1	5,92	1,666	0,00	0,00	28,96	2,41	3,24	3,07	3,54	0,00	0,00	0,00
18	5,74	3,044	1,34	5,55	22,03	0,00	2,51	2,94	0,84	0,00	0,82	0,00
42	5,63	3,015	1,34	15,03	11,37	0,00	1,67	2,71	0,49	0,00	1,93	0,21
66,25	5,66	3,340	1,20	19,40	5,78	0,00	1,51	2,78	0,00	0,00	2,78	0,25
71,25	5,67	3,360	1,27	20,05	4,85	0,00	1,38	2,76	0,00	0,00	2,82	0,31
71,5	5,56	3,505	0,98	16,67	98,43	0,25	0,00	12,27	0,00	0,00	2,57	0,23
138	5,31	4,790	0,79	34,60	73,06	0,00	0,00	12,15	0,00	0,00	6,24	0,71
162,5	5,26	4,708	0,67	39,74	64,03	0,00	0,00	11,78	0,00	0,00	7,64	0,87
186,25	5,12	4,716	0,60	46,09	57,30	0,00	0,00	11,81	0,00	0,00	9,22	1,06
210,5	5,09	4,526	0,45	51,80	49,32	0,00	0,00	11,63	0,00	0,00	10,50	1,29
234,25	5,04	4,636	0,37	57,95	41,18	0,00	0,00	11,52	0,00	0,00	11,82	1,53
307	4,75	4,864	0,00	69,07	19,89	0,00	0,00	10,21	0,00	0,00	14,42	2,29
335,25	4,62	4,946	0,00	72,08	13,82	0,00	0,00	9,65	0,00	0,00	15,78	2,72
355,75	4,57	4,766	0,00	76,00	10,42	0,00	0,00	9,54	0,00	0,00	16,89	3,22
361,5	4,57	5,010	0,00	74,68	9,09	0,00	0,00	9,09	0,00	0,00	16,94	3,20
361,5	4,57	5,010	CDM = 1,01 g/L						**0,300 g CDM/L/OD**			

9.9 Raw data of the fermenter runs with hydrolysate-medium

Appendix table 60: C238 hydrolysate-medium B (run 1)

Time [h]	pH	OD_{600}	OD-T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,89	0,896	0,896	0,00	0,00	28,33	8,73	3,88	0,77	0,04	0,00
1	5,86	1,390	0,494	0,00	0,00	27,56	8,57	3,68	0,78	0,04	0,00
17,5	5,76	5,338	4,442	2,70	0,33	26,95	0,00	3,41	0,75	0,43	0,04
41,5	5,75	6,014	5,118	2,67	0,98	25,60	0,00	3,53	0,76	0,45	0,08
65,75	5,68	6,824	5,928	2,65	2,66	22,59	0,00	3,44	0,75	0,49	0,11
138	5,73	8,586	7,690	2,65	10,88	10,74	0,00	3,31	0,66	1,02	0,36
162,25	5,63	11,068	10,172	2,66	13,15	6,65	0,00	3,09	0,59	1,21	0,46
186	5,53	13,004	12,108	2,70	14,24	4,07	0,00	2,58	0,54	1,50	0,53
210	5,48	15,828	14,932	2,56	14,20	0,00	0,00	2,04	0,48	1,40	0,63
210,5	5,32	11,884	10,988	2,16	10,72	91,87	30,88	14,38	0,36	1,15	0,45
234,25	4,97	23,600	22,704	14,80	13,51	75,54	0,00	12,99	0,29	3,79	1,15
306	4,84	25,010	24,114	15,53	21,16	73,41	0,00	13,31	0,15	3,90	1,76
330,5	4,87	25,180	24,284	15,63	23,16	66,59	0,00	13,15	0,08	3,80	1,86
354,25	4,87	24,620	23,724	16,16	27,18	66,23	0,00	14,09	0,06	3,97	2,07
378,5	4,92	24,760	23,864	16,42	29,65	60,23	0,00	13,24	0,04	3,99	2,18
402,5	4,98	23,510	22,614	16,53	33,24	56,92	0,00	13,42	0,00	4,11	2,32
404,5	4,99	24,260	23,364	16,39	32,33	55,25	0,00	13,18	0,00	4,03	2,24
402,5	4,98	23,510	CDM = 5,58 g/L		0,237 g CDM/L/OD		mean:	**0,239**	**g CDM/L/OD**		
			CDM = 5,68 g/L		0,242 g CDM/L/OD						
404,5	4,99	24,260	CDM = 5,94 g/L		0,245 g CDM/L/OD		mean:	**0,245**	**g CDM/L/OD**		
			CDM = 6,12 g/L		0,252 g CDM/L/OD						
			CDM = 5,80 g/L		0,239 g CDM/L/OD						

Appendix table 61: C238 hydrolysate-medium B (run 2)

Time [h]	pH	OD_{600}	OD-T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,84	0,637	0,637	0,00	0,00	35,27	10,93	4,96	1,07	0,04	0,00
1,25	5,81	1,314	0,677	0,52	0,00	35,12	10,57	4,62	1,09	0,10	0,00
19,25	5,61	5,413	4,776	3,33	0,52	32,92	0,00	4,29	1,06	0,61	0,00
42,5	5,43	7,050	6,413	3,34	1,91	30,66	0,00	4,27	1,02	0,64	0,00
65,75	5,57	8,050	7,413	3,32	5,03	26,17	0,00	4,37	1,04	0,75	0,14
139	5,24	13,736	13,099	4,36	12,56	11,26	0,00	3,64	0,26	0,11	0,31
164,75	5,28	14,932	14,295	4,93	12,87	8,16	0,00	3,63	0,45	0,09	0,36
166,5	5,15	12,812	12,175	3,95	9,85	100,22	33,44	16,71	0,37	0,08	0,23
185	4,85	19,240	18,603	11,89	10,03	95,84	15,00	15,84	0,50	1,54	0,43
Experiment stopped, strong bacterial contamination.											
164,75	5,28	14,295	CDM = 4,76 g/L				mean:	**0,238**	**g CDM/L/OD**		

Appendix table 62: C245 hydrolysate-medium A (run 1)

Time [h]	pH	OD_{600}	OD-T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,89	0,898	0,898	0,00	0,00	28,48	9,10	3,74	0,97	0,03	0,00
1	5,89	1,488	0,590	0,00	0,00	28,59	9,00	3,85	0,95	0,05	0,00
17,5	5,66	4,030	3,132	1,76	0,41	26,93	2,18	4,06	0,89	1,00	0,11
41,5	5,68	4,240	3,342	1,96	6,84	18,22	0,00	3,70	0,96	2,01	0,18
65,75	5,55	2,928	2,030	1,86	9,85	11,91	0,00	3,28	0,91	2,91	0,34
137,75	5,18	5,530	4,632	0,63	9,69	5,83	0,00	3,20	0,80	2,91	0,20
162	4,99	2,554	1,656	0,22	9,41	4,17	0,00	2,78	0,69	2,63	0,19
185,75	4,77	4,951	4,053	0,00	9,12	2,83	0,00	2,43	0,74	2,50	0,25
186,25	4,83	20,084	19,186	0,00	6,80	90,29	31,43	16,09	0,45	1,96	0,17
209,75	4,25	29,710	28,812	12,10	11,85	77,05	0,00	12,92	0,28	5,39	1,00
234	4,38	35,950	35,052	12,51	13,78	85,80	0,00	14,15	0,32	5,84	1,21
305,75	4,18	30,040	29,142	13,69	28,37	46,93	0,00	12,28	0,09	5,82	3,17
330,25	4,13	30,520	29,622	13,77	31,52	35,67	0,00	12,09	0,00	5,70	3,97
354	4,20	28,260	27,362	14,05	36,82	25,38	0,00	11,84	0,00	5,84	4,87
378,25	4,28	27,700	26,802	14,55	41,58	15,90	0,00	11,65	0,00	5,93	5,91
402,25	4,30	25,880	24,982	14,69	42,99	9,32	0,00	11,36	0,00	5,63	6,75
404,75	4,31	39,180	38,282	14,70	42,55	8,43	0,00	11,24	0,00	5,94	6,83
402,25	4,30	25,880	CDM = 4,76 g/L		0,184	g CDM/L/OD	mean:	**0,191**	**g CDM/L/OD**		
			CDM = 5,14 g/L		0,199	g CDM/L/OD					
404,75	4,31	39,18	CDM = 9,52 g/L		0,243	g CDM/L/OD	mean:	**0,247**	**g CDM/L/OD**		
			CDM = 9,74g/L		0,249	g CDM/L/OD					
			CDM = 9,76 g/L		0,249	g CDM/L/OD					

Appendix table 63: C245 hydrolysate-medium A (run 2)

Time [h]	pH	OD_{600}	OD-T0	Ethanol [g/L]	Xylitol [g/L]	Xylose [g/L]	Glucose [g/L]	Arabinose [g/L]	Acetate [g/L]	Glycerol [g/L]	Arabitol/Mannitol [g/L]
0	5,80	0,702	0,702	0,00	0,00	35,37	10,81	4,71	1,16	0,05	0,00
1,25	5,74	1,298	0,597	0,00	0,00	35,07	10,67	4,76	1,13	0,07	0,00
19,25	5,52	3,213	2,511	2,02	0,23	34,24	3,16	4,78	1,15	1,20	0,09
42,5	5,28	2,172	1,470	2,36	2,13	27,98	0,00	4,53	1,15	2,13	0,52
65,75	5,20	3,793	3,091	2,14	3,62	22,01	0,00	4,39	1,08	2,40	0,83
139	5,03	15,200	14,498	2,48	11,28	6,80	0,00	3,67	0,89	2,99	1,29
164,75	5,94	13,676	12,974	2,04	11,33	0,00	0,00	3,40	0,66	2,92	0,95
166,5	4,88	16,032	15,330	5,03	8,66	98,06	33,23	15,96	0,48	2,77	0,65
186,5	4,47	18,850	18,148	13,49	11,44	90,79	0,00	15,33	0,41	5,21	2,02
210,75	4,42	19,720	19,018	13,91	17,84	76,32	0,00	14,36	0,30	5,23	2,61
233,75	4,30	18,800	18,098	14,23	23,59	66,67	0,00	14,29	0,24	5,32	3,15
306	4,44	23,464	22,762	15,14	37,02	42,83	0,00	13,64	0,00	5,42	4,67
330,25	4,43	19,656	18,954	15,49	39,82	35,33	0,00	13,73	0,00	5,46	5,00
354	4,18	19,216	18,514	16,10	44,32	27,31	0,00	13,65	0,00	5,58	5,67
378,25	4,29	25,160	24,458	16,70	45,47	24,53	0,00	13,47	0,00	5,67	5,88
401,75	4,18	21,416	20,714	17,24	47,08	17,61	0,00	12,55	0,00	5,81	6,22
474,5	4,09	22,248	21,546	17,30	51,43	4,56	0,00	10,78	0,00	5,55	7,79
475,75	4,08	34,208	33,506	17,17	50,41	4,52	0,00	10,68	0,00	5,44	7,70

474,5	4,09	22,248	CDM = 5,64 g/L	0,254	g CDM/L/OD	mean:	**0,253**	**g CDM/L/OD**
			CDM = 5,66 g/L	0,254	g CDM/L/OD			
			CDM = 5,58 g/L	0,251	g CDM/L/OD			
475,75	4,08	34,208	CDM = 10,12 g/L	0,296	g CDM/L/OD	mean:	**0,283**	**g CDM/L/OD**
			CDM = 9,42 g/L	0,275	g CDM/L/OD			
			CDM = 9,46 g/L	0,277	g CDM/L/OD			