Exploitation of Wheat Straw Biorefinery Side Streams as Sustainable Substrates for Microorganisms: A Feasibility Study

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Keywords: tetraether, lipids, polyhydroxybutyrate, acetic acid, side streams, carbohydrates, liquid hot water, biorefinery, lignocellulose

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Lignocellulosic agricultural side products, like wheat straw, are widely seen as an important contribution to a future sustainable economy. However, optimization of biorefinery processes and exploitation of all side streams are crucial for an economically viable biorefinery. Pretreatment of lignocellulosic raw material, which is necessary for further processing steps, can generate low-value side streams. In this feasibility study, side streams from a liquid hot water (LHW) pretreatment of wheat straw were utilized for the production of polyhydroxybutyrate (PHB) and highly valuable tetraether lipids (TELs). Additional value created by these products can benefit the biorefinery’s economic operation. The utilized wheat straw was pretreated at 120 °C and 170 °C for up to two hours in laboratory and lab scale. The resulting side stream consists mainly of carbohydrates from hemicelluloses and fermentation inhibitors such as acetic acid. In order to achieve a successful production of both products, an acetic acid separation via distillation was necessary. Subsequently, the acetic acid fraction was utilized for the PHB production using cyanobacteria. The carbohydrate-rich fraction was applied in the cultivation of Sulfolobus acidocaldarius and resulted in the successful production of TELs. Both fractions achieved better fermentation yields compared to their corresponding reference media.

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Abstract: Lignocellulosic agricultural side products, like wheat straw, are widely seen as an important contribution to a future sustainable economy. However, optimization of biorefinery processes and exploitation of all side streams are crucial for an economically viable biorefinery. Pretreatment of lignocellulosic raw material, which is necessary for further processing steps, can generate low-value side streams. In this feasibility study, side streams from a liquid hot water (LHW) pretreatment of wheat straw were utilized for the production of polyhydroxybutyrate (PHB) and highly valuable tetraether lipids (TEls). Additional value created by these products can benefit the biorefinery’s economic operation. The utilized wheat straw was pretreated at 120 °C and 170 °C for up to two hours in laboratory and lab scale. The resulting side stream consists mainly of carbohydrates from hemicelluloses and fermentation inhibitors such as acetic acid. In order to achieve a successful production of both products, an acetic acid separation via distillation was necessary. Subsequently, the acetic acid fraction was utilized for the PHB production using cyanobacteria. The carbohydrate-rich fraction was applied in the cultivation of Sulfolobus acidocaldarius and resulted in the successful production of TELs. Both fractions achieved better fermentation yields compared to their corresponding reference media.

Keywords: lignocellulose; biorefinery; liquid hot water; side streams; carbohydrates; acetic acid; polyhydroxybutyrate; lipids; tetraether

1. Introduction

The world is currently confronted with the progressive depletion of its resources, mainly based on non-renewable feedstocks [1]. Lignocellulosic biomass residues are estimated to exceed 2 × 10¹¹ t/year worldwide and offer a vast source for the production of renewable products and chemicals in so-called biorefineries [2,3]. In order to achieve an economic operation and efficient processing of renewable feedstocks into bio-based products, all biomass fractions are supposed to be turned into marketable products [4].

A biorefinery approach involves a multi-step process in which the first step, subsequent to the feedstock selection, typically involves treating the biomass to pre-separate the main components, that is, cellulose, hemicellulose, and lignin, and to make it more amenable for further processing [5]. This step is conventionally referred to as pretreatment and can account for up to 20%–40% of the overall production costs, which puts a special focus on its optimization [6].

Among the various pretreatment methods, hydrothermolysis using water at elevated temperature and under pressure has shown to be effective in removing and solubilizing hemicellulose, thus
improving the subsequent processing. The so-called liquid hot water (LHW) pretreatment of lignocellulosic biomass dissolves hemicelluloses and some of the lignin while minimizing the formation of inhibitors for subsequent fermentation processes [7]. The process results in a solid stream containing mainly cellulose and lignin and an aqueous side stream containing mainly dissolved hemicelluloses and acetic acid. In terms of the improvement of the process economics, mentioned components in the aqueous side stream can potentially be used as carbon source for microbial fermentation processes. In this feasibility study, we exemplarily tested the utilization of this aqueous side stream as substrates for the cultivation of cyanobacteria producing polyhydroxybutyrate (PHB; e.g., [8,9]) as well as for the cultivation of Sulfolobus acidocaldarius, a thermoacidophilic archaean and producer of highly valuable tetraether lipids (TELs; e.g., [10]). We chose these two very different hosts due to the synergies that emerge from the combination of the hydrolysate and the organisms: S. acidocaldarius can directly grow on the hot and acidic hydrolysate without the need for extensive cooling and neutralization, while the photoautotrophic cyanobacteria can thrive on acetate as additional energy source. At the same time, S. acidocaldarius benefits from the removal of acetate since it is a potent growth inhibitor for the archaean.

PHB is the best-characterized member of the polyhydroxyalkanoate (PHA) family and is widespread in various bacterial species as storage material [11,12]. PHB is biodegradable (and compostable according to EN 13432), insoluble in water, non-toxic, and biocompatible. Therefore, PHB could be an attractive alternative to petroleum-based plastics [13]. It resembles the commodity polymer polypropylene in its properties [14]. PHB is commercially produced by heterotrophic bacteria such as Alcaligenes eutrophus [15], Alcaligenes latus [16], and recombinant Escherichia coli [17]. Despite relatively high yields of PHB, production from bacterial fermentation requires sugar supplementation and continuous oxygen supply that results in high substrate and operation costs [11,18]. In addition, public discussion about bioplastics production from sugar feedstocks is similar to the discussion about first-generation biofuels. Competition of material with food and feed production for the same resources potentially leads to shortages and price increases and is also contributing to climate change through direct and indirect land use change [19].

TELs, the major membrane constituents of S. acidocaldarius, are gaining rapidly increasing attention as a unique biological material for pharmaceutical applications [20–24]. These TELs are highly stable at elevated temperatures and low pH values and resent towards enzymatic degradation. TELs are mainly used in medical applications for the formation of drug delivery vehicles. Despite the numerous promising publications on the pharmaceutical utilization of TELs, no commercial applications exist to date. Reason for that are too high production costs and low availability of the lipids. As expenditures for cultivation media are the most important cost driver in microbial pharmaceutical bioprocesses, this problem was tackled by investigating the use of a cheap and sustainable alternative carbon source to supplement or replace routinely used carbon sources, like glucose or NZ-amine, as substrate for S. acidocaldarius. This role can be fulfilled especially well by substrates based on biomass waste streams from forestry, food or agricultural industries, like the here investigated agricultural by-product wheat straw. To date, no cultivations of S. acidocaldarius on lignocellulosic hydrolysates have been published in the literature. Established cultivation media for Sulfolobus species are based on protein hydrolysates, like yeast extract or casein hydrolysate (e.g., Schiraldi et al. [25]). Also defined media, containing glutamate as primary carbon source [26], and growth on whey, containing lactose as primary carbon source [27], have been described for Sulfolobus species. Likewise, to our knowledge there are no reports on the use of acetate derived from lignocellulosic hydrolysates for the cultivation of cyanobacteria.

This feasibility study shows the pretreatment of the residual lignocellulose wheat straw in an LHW process in laboratory and pilot scale. The resulting liquid side streams containing hemicellulose and acetic acid were investigated in terms of their suitability for the recombinant production of PHB and TELs in laboratory experiments.
2. Materials and Methods

2.1. Materials

The wheat straw used was harvested in 2015 in lower Austria and stored under dry conditions until use. The composition is given in Table 1.

<table>
<thead>
<tr>
<th>Arabinan 1</th>
<th>Galactan 1</th>
<th>Cellulose 1,2</th>
<th>Xylan 1</th>
<th>Mannan 1</th>
<th>Lignin</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.68 ± 0.0717</td>
<td>0.872 ± 0.0360</td>
<td>32.5 ± 0.985</td>
<td>19.8 ± 0.432</td>
<td>0.409 ± 0.00937</td>
<td>16.1 ± 0.518</td>
<td>0.539 ± 0.261</td>
</tr>
</tbody>
</table>

1 Measured in hydrolyzed form but given as their non hydrolyzed counterparts; 2 Based on glucose content.

2.2. Process Description

The entire process can be divided into three main steps which are illustrated in Figure 1. Wheat straw is pretreated, and the resulting liquid fraction is separated in a carbohydrate rich fraction and an acetic acid rich fraction. Subsequently, the carbohydrate fraction is used for the cultivation of *S. acidocaldarius* and the acetic acid fraction for the cultivation of cyanobacteria for the production of TELs and PHB, respectively. These main steps are subsequently described in detail.

![Schematic description including main process steps pretreatment, fractionation and cultivation.](image)

**Figure 1.** Schematic description including main process steps pretreatment, fractionation and cultivation.

2.3. Wheat Straw Pretreatment

Liquid hot water (LHW) treatment applies only water at elevated temperatures in liquid state to pretreat the wheat straw. Experiments were conducted in lab-scale and pilot-scale equipment.

The lab-scale LHW experiments were conducted in 45 mL pressure vessels (PARR, Model 4716, Moline, IL, USA) in electrical heating jackets without stirring. Around 2.3 g of wheat straw and the according amount of water were added to the pressure vessels to achieve a 9.2 wt % wheat straw content. The reactors were heated within 10 min to 120 °C and 170 °C, respectively, and held at this temperatures for the desired time of 0, 30, 60, 90, and 120 min. Afterwards, the pressure vessels were immersed in a cooled water bath in order to achieve fast cooling of the reactor content, and the solid and liquid phases were separated for analysis. The pretreatments in lab-scale and extract analysis were performed in duplicate for all conditions.

The pilot-scale LHW experiments were conducted in a 10 l DIG-MAZ extraction system (Samtech Extraction Systems, Mannersdorf, Austria). In brief, the extraction plant consists of a jacketed fixed bed reactor and water is circulated through the bed of wheat straw. Identical to the lab-scale experiments, the wheat straw content was around 9.2 wt % and the extraction temperature was 120 °C. Water was preheated to around 100 °C before injection in the reactor. Therefore, similar temperature gradients compared to the lab-scale experiments were achieved. Extract samples were taken after 0, 30, 60, 90, and 120 min. The pretreatment in pilot-scale was repeated six times and extract analysis was performed in duplicate. The pilot-scale extracts were then concentrated by a factor of 5 in a thin
film evaporator unit of the DIG-MAZ extraction system (Samtech Extraction Systems, Mannersdorf, Austria). The surface temperature was set to 95 °C and the pressure to 70 mbar.

2.4. Extract Separation

Acetic acid was separated in a rotary evaporator R-220 EX (Buchi, Flawil, Switzerland) by lowering the pH value to around 3 with sulfuric acid. The separation was conducted in 5 steps, the nonvolatile fraction was filled up to the initial volume with water and the pH value was set back to 3 after each individual step. The heating bath temperature was set to 57 °C and the pressure to 60 mbar.

The acetic acid-containing distillate was concentrated by neutralizing the pH value to 7 with sodium hydroxide and the separation of water in the before mentioned setup.

2.5. Cultivation of S. acidocaldarius

*Sulfolobus acidocaldarius* DSM 639 was grown aerobically at 75 °C in a reciprocal shaking oil bath at 100 rpm in 100 mL long-neck Erlenmeyer flasks to avoid evaporation. The flasks were filled with 20 to 50 mL LHW extract or culture medium and the initial pH was adjusted to 3.0 with 4.8 wt % H₂SO₄. Unless stated otherwise, salts and trace elements were provided by addition of appropriate stock solutions as described elsewhere. The medium described by Brock et al. [28] was used as reference for the evaluation of biomass growth. All measurements of biomass concentration and the cultivation were performed in duplicates.

2.6. Cultivation of Cyanobacteria

*Synechocystis* sp. PCC 6714 was grown in basal BG-11 medium [29] with 10 mM HEPES buffer, pH 8.5. The medium was either supplemented with the acetic acid fraction from the distillation step, or, in case of the reference medium, with 5 mM NaHCO₃ as carbon source.

All measurements of biomass concentration were performed in duplicates, the fermentation was performed as single experiment.

2.7. Analytics

The carbohydrate content was determined by the sample preparation following the National Renewable Energy Laboratory (NREL) laboratory analytical procedure (LAP), “Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples” [30], but no neutralization of the samples after hydrolysis was conducted and a Thermo Scientific ICS-5000 HPAEC-PAD system (Thermo Scientific, Waltham, MA, USA) with deionized water as the eluent was used for the determination of arabinose, glucose, mannose, xylose, and galactose on a CarboPac PA1 (Dionex, USA) column. The concentration of the degradation products acetic acid, hydroxymethylfurfural (HMF), and furfural, was determined with a Shimadzu HPLC system and a Shodex SH1011 column (Showa Denko, Tokyo, Japan) at 40 °C, with 0.005 M H₂SO₄ as eluent.

The yields of the carbohydrates in the pretreatment process are defined as:

\[
Yield_{\text{carbohydrates}}(\%) = \frac{m_{\text{carbohydrate}}(g)}{m_{\text{carbohydrate in wheat straw}}(g)} \cdot 100(\%)
\]

where \(m_{\text{carbohydrate}}\) is mass of dissolved carbohydrates in the extract and \(m_{\text{carbohydrate in wheat straw}}\) is the mass of the corresponding carbohydrate in the initial wheat straw. In case of total carbohydrates, the sum of monomers and oligomers of arabinose, galactose, glucose, xylose and mannose is used.

The yields of the degradation products acetic acid, furfural and HMF in the pretreatment process are defined as:

\[
Yield_{\text{degradation}}(\%) = \frac{m_{\text{degradation product}}(g)}{m_{\text{wheat straw}}(g)} \cdot 100(\%)
\]
where $m_{\text{degradation product}}$ is the total mass of the resulting of a certain degradation product and $m_{\text{initial dry matter of wheat straw}}$ is the initial dry matter of wheat straw.

Microbial growth was monitored with a photometer (Genesys 20 photometer (Thermo Scientific, Waltham, MA, USA) via determination of the optical density at 600 nm and 750 nm for *S. acidocaldarius* and *Synechocystis* sp. PCC 6714, respectively.

3. Results & Discussion

3.1. Wheat Straw Pretreatment

Wheat straw needs to be pretreated in order to pre-separate the main components of the biomass and to make it more amenable for further processing [5]. In this study, LHW was used as the pretreatment method. Thereby, only water at elevated temperatures is used. Factors influencing the pretreatment severity include reaction temperature and time. On the one hand, higher pretreatment temperatures and time increase the efficiency, but on the other hand, they also increase the concentration of degradation products which are potential inhibitors for subsequent fermentation processes [31,32]. The influence of the pretreatment on the solid fraction and its enzymatic hydrolysis yields is well investigated [33–35] and was not aimed in this study. Figure 2 shows the carbohydrate solubilization yields of the pretreatments in laboratory and pilot scale. Increasing temperatures shows a sharp increase of the carbohydrate yields. The yield is increasing from 3.5% to 24% after 90 min pretreatment time for the 120 °C and 170 °C lab-scale experiment, respectively.

![Figure 2](image_url) **Figure 2.** Total carbohydrate yields during the liquid hot water (LHW) pretreatments.

Comparing the carbohydrate monomer percentage on the total carbohydrates dissolved during the process, the monomer percentage in the 120 °C pilot-scale pretreatments was 22.6 ± 2.2% and decreased to 14.1 ± 5.2% in the 170 °C laboratory-scale experiments. Changes of the monomer percentages were not evident over the pretreatment time. Higher monomer concentrations are favorable for subsequent microbial fermentation steps since otherwise required additional enzymatic hydrolysis steps can be avoided.

Figure 3 shows the average distributions of the carbohydrates (arabinose, galactose, xylose, glucose, and mannose) in the pretreatment extracts of the pilot-scale pretreatment at 120 °C and the lab-scale pretreatment at 170 °C. The predominant carbohydrate species at the pretreatment temperature of 120 °C was glucose with 57.3%. However, at 170 °C pretreatment temperature, xylose
showed a share of 50.8%. Glucose is a hexose, whereas xylose is a pentose, and subsequent fermentation steps based on classical biotechnological hosts are likely to be suitable for only one of the carbohydrate types due to the phenomenon of carbon catabolite repression. Therefore, pretreatment temperature can have a significant influence on subsequent process steps and needs to be considered. However, a pretreatment duration dependency was not evident.

![Figure 3](attachment:figure3.png)

**Figure 3.** Average total carbohydrate distributions over the pretreatment time in the extracts of the pilot-scale pretreatment at 120 °C and the lab-scale pretreatment at 170 °C.

However, increasing the temperature also increased the production of degradation products, acetic acid, hydroxymethylfurfural (HMF), and furfural, which can inhibit subsequent microbial fermentation processes [36–38]. Acetic acid originates from the cleavage of the acetyl linkages in xylan and presumably also from hydrolytic cleavage of the acetyl substitutions in, for example, acetylated galacto-glucomannans. HMF and furfural are generated at elevated temperatures from xylose and glucose, respectively [37]. The pretreatment temperature and time dependency of the quantitatively highest degradation product, acetic acid, is shown in Figure 4B. Acetic acid yields were increasing over pretreatment duration under all tested pretreatment conditions. However, the temperature had the main influence on the acetic acid formation. Comparing the acetic acid yields after 120 min pretreatment, the temperature increase from 120 °C to 170 °C led to an approximate 3.4-fold increase of the acetic acid formation. Similar trends can be seen in the formation of furfural and HMF. The time dependency of their formation at 170 °C pretreatment temperature is shown in Figure 4A. Longer pretreatment increases the formation of both HMF and furfural, whereas the latter is the predominant of the mentioned species. However, these components were not detectable at pretreatment temperatures of 120 °C.

![Figure 4](attachment:figure4.png)

**Figure 4.** (A) Acetic acid generated during the LHW pretreatment. (B) Degradation product formation during the lab-scale pretreatments at 170 °C. Shown degradation products were below detection limit (1.3 mg/L for Furfural; 0.6 mg/L for HMF) at pretreatments at 120 °C.
In order to achieve higher carbohydrate concentrations in the extract, it was concentrated in a thin film evaporator by a factor of 5.04. The composition of the extract after the concentration step is shown in Table 2.

### Table 2. Composition of the concentrated wheat straw extract.

<table>
<thead>
<tr>
<th>Arabinose</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Xylose</th>
<th>Mannose</th>
<th>Acetic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomeric Carbohydrates (g/L)</td>
<td>0.549</td>
<td>0.176</td>
<td>5.32</td>
<td>0.459</td>
<td>0.533</td>
</tr>
<tr>
<td>Total Carbohydrates (g/L)</td>
<td>0.833</td>
<td>0.953</td>
<td>9.03</td>
<td>2.17</td>
<td>0.732</td>
</tr>
<tr>
<td>Degradation Products 1 (g/L)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

1 HMF and Furfural were below detection limit.

### 3.2. Fractionation of the Extract

The high acetic acid content of the extract caused growth inhibition of *S. acidocaldarius* when the extract was directly used as carbon source. Therefore, acetic acid was separated using distillation under acidic conditions. The separated aqueous acetic acid solution was subsequently concentrated in rotary evaporator at neutral pH values. The process resulted in two fractions—an acetic acid-rich fraction and a carbohydrate fraction. The composition of the latter is shown in Table 3 and the acetic acid fraction reached an acetic acid concentration of 3.11 g/L.

### Table 3. Composition of the carbohydrate fraction.

<table>
<thead>
<tr>
<th>Arabinose</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Xylose</th>
<th>Mannose</th>
<th>Acetic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomeric Carbohydrates (g/L)</td>
<td>1.22</td>
<td>0.090</td>
<td>3.67</td>
<td>0.0860</td>
<td>0.254</td>
</tr>
<tr>
<td>Total Carbohydrates (g/L)</td>
<td>2.39</td>
<td>2.08</td>
<td>11.4</td>
<td>2.56</td>
<td>0.664</td>
</tr>
<tr>
<td>Degradation Products 1 (g/L)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

1 HMF and Furfural were below detection limit.

### 3.3. Cultivation of *S. acidocaldarius*

While *S. acidocaldarius* was initially described as sulfur oxidizing autotroph [28], the today commercially available strains are strictly heterotrophs that thrive best on protein hydrolysates, but also sugars and single amino acids can serve as substrates [39]. Although present, reports of bioreactor cultivations of *S. acidocaldarius* are scarce [10]. Typically, and similar to the experiments performed in this study, the organism is cultivated in shake flasks in small scales.

In the present study, in order to lower the inhibitor concentrations after the acetic acid removal, the carbohydrate faction of the LHW extract was diluted 1:2. However, growth of *S. acidocaldarius* was still not possible on the medium. Due to the already low content of fermentable sugars, further dilution was not considered a feasible option and, therefore, the substrate was detoxified by incubation with 5 wt % activated carbon (AC) at 50 °C for 75 min to reduce the concentration of potential inhibitors. The sugar content of the 1:2 diluted carbohydrate fraction before and after the treatment with AC is shown in Table 4. When supplemented with only 0.5 g/L NZ-amine as additional carbon source, the AC-treated hydrolysate supported a growth rate of *S. acidocaldarius* similar to a reference medium containing 2 g/L glucose and 1 g/L NZ-amine until growth ceased at an approximate OD$_{600}$ of 1.2 due to nutrient depletion (Figure 4).

### Table 4. Monomeric Carbohydrate content before and after detoxification with activated carbon (AC).

<table>
<thead>
<tr>
<th>mg/L</th>
<th>Arabinose</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Xylose</th>
<th>Mannose</th>
<th>Acetic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before AC Treatment</td>
<td>609</td>
<td>45</td>
<td>1835</td>
<td>43</td>
<td>127</td>
<td>64</td>
</tr>
<tr>
<td>After AC Treatment</td>
<td>167</td>
<td>25</td>
<td>1534</td>
<td>-</td>
<td>41</td>
<td>56</td>
</tr>
</tbody>
</table>
Figure 5 shows that wheat straw hydrolysate could be successfully used to replace the two carbon sources, glucose and NZ-amine, contained in a reference medium to a large part. However, in order to use wheat straw hydrolysate, the separation of acetate and the removal of inhibitors were necessary steps.

![Graph showing growth rate comparison](image)

**Figure 5.** Time dependent growth of *S. acidocaldarius* in reference medium and LHW carbohydrate fraction detoxified with activated carbon (AC).

### 3.4. Cultivation of Cyanobacteria

Although the cultivation of cyanobacteria can be carried out in natural locations as well as in artificial open pond systems [40], the desired production system in an industrial setup is the controlled environment of a photobioreactor (PBR). With these systems it is possible to obtain defined and reproducible cultivation conditions. PBR are capable of providing a wide range of process parameters (e.g., pH, temperature, light intensity, nutrient supply, mixing time, etc.) and can therefore be adapted easily for any desired cyanobacteria species. In the present study we also employ controlled conditions regarding nutrient supply, initial pH, temperature, light intensity and mixing. For efficient PHB production cyanobacteria require significant amounts of nutrients, such as nitrogen, phosphorus, sulfur, potassium, magnesium and iron. Besides media components, other key parameters influencing growth and PHB production in cyanobacteria are cultivation conditions, such as temperature, pH, light intensity, or light/dark cycles [8]. Another strategy for generation of increased amounts of cyanobacterial biomass is the addition of acetate to the fermentation broth. This approach has been shown to be successful in laboratory scale using commercially available acetate [41,42].

In the present study PHB-producing cyanobacteria were cultivated on a medium containing 3.1 g/L acetate from the distillation step. Microbial growth was boosted in comparison with the reference medium containing only an inorganic carbon source (5 mM NaHCO₃) as described by Kamravamanesh et al. [43].

As shown in Figure 6, the addition of acetate led to an increased growth rate compared to a reference medium without an organic carbon source.
4. Conclusions

A complete process chain starting from wheat straw pretreatment using a liquid hot water process, the fractionation of the pretreatment waste stream and the successful utilization of these fractions for the cultivation of PHB- and TEL-producing microorganisms was shown. Severe pretreatment conditions led to higher concentrations of degradations products such as acetic acid, furfural, and HMF. Whereas it was shown that acetic acid can be separated from the pretreatment waste stream and used as carbon source for cyanobacteria, the higher concentrations of the degradation products made a further detoxification step necessary for the cultivation of *S. acidocaldarius*. Both cultivations obtained by applying lignocellulosic biorefinery side streams were found to be feasible. This opens the opportunity for the improvement of the economics of a biorefinery by through the production of high value products from low-value side streams and leads direction to an intended 100% usage of the applied raw material.

Author Contributions: S.B. designed and performed the pretreatment experiments. J.Q. designed and performed the cultivation of *S. acidocaldarius*. D.K. designed and performed the cultivation of cyanobacteria. S.B., J.Q., and O.S. wrote and edited the manuscript with significant input and editing from D.K. and A.F.

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Conflicts of Interest: The authors declare no conflict of interest.

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