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UPCYCLING OF PAPER TOWEL WASTE – ENZYMATIC SACCHARIFICATION FOR A CIRCULAR ECONOMY

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ABSTRACT

The increasing demand for renewable carbon sources and the depletion of fossil resources highlight the need for sustainable solutions in the chemical industry. This study evaluates the potential of used paper towels as a feedstock for glucose production and subsequent biotechnological applications. A paper towel sorting and processing concept was developed for a model municipality. Pilot scale sample sorting was conducted to assess practical feasibility and impurity content. Targeted interventions in public facilities led to impurity levels below 1% (w/w) in some samples. Enzymatic saccharification with commercial enzyme blends achieved yields of up to 98.4% with unused paper towels under optimized laboratory conditions. At pilot scale, saccharification reached yields of up to 57%, mainly limited by mixing dynamics and microbial contamination. Sanitization reduced contaminants but did not completely prevent environmental microbial growth, leading to a reduction in saccharification time from 48 h to 24 h to improve energy efficiency. The resulting glucose syrups were tested as the main carbon source for the growth of five model microorganisms (*Saccharomyces cerevisiae, Escherichia coli, Lactobacillus rhamnosus, Cupriavidus necator* and *Penicillium verruculosum*). The results demonstrate that glucose from upcycled paper towels is broadly suitable and, in some cases, superior to laboratory-grade glucose for microbial fermentation, as the obtained syrups contain additional fermentable sugars, highlighting a promising pathway for sustainable carbon sourcing in the bioeconomy.

1. INTRODUCTION

Considering the global challenges posed by diminishing fossil resources and advancing climate change, bioeconomy has become increasingly vital for fulfilling the carbon demands of the chemical industry through the combination of a circular economy and upcycling processes (Verma & Jones, 2025). Key elements include carbon capture and utilization, recycling streams and biomass, focusing especially on the multi-use and long-term carbon sequestration of biogenic residues and waste materials (European Commission, 2020, 2025).

Among biomass streams, municipal solid waste is a material stream that largely avoids conflicts of use (fuel versus food) (Langsdorf et al., 2021; Volkmar et al., 2023). Due to its high proportion of over 94% of plant-based biomass, lignocellulosic biomass is a very powerful and primary resource (Pietzsch, 2017, p. 80). This natural composite of lignin, hemicellulose and cellulose enables numerous value chains and products from non-food feedstocks such as biofuels, biosurfactants, organic acids and other chemical products (Du et al., 2026; Sulis et al., 2025; Volkmar et al., 2023). Their efficient production requires the separation of the three components, for which a series of pretreatment steps have been established (Nair & Verma, 2025; Segers et al., 2024). Due to this structural complexity, the focus for material utilization is on cellulose, as cellulose not only constitutes the largest proportion of plant biomass but, unlike the other two components, is a polymer with a regular structure that is enzymatically broken down by cellulases into its glucose monomers (Usmani et al., 2021). For the saccharification and biotechnological conversion of sugars from lignocellulosic feedstocks, main processes are distinguished as separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) or consolidated bioprocessing (CBP) according to the sequence of process steps (Verardi

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et al., 2023). The enzymes required for the lignocellulosic degradation can be added from an external source or simultaneously produced by suitable microorganisms on the same feedstock base (Jahangeer et al., 2025).

The feedstock selected in this study, paper towels, combines the characteristics of a widely available, largely unused fraction of the residual waste stream with a favorable material composition. Due to a comparatively homogeneous composition and a low-lignin content, as most of the lignin has already been removed during the pulping process, complex treatment steps are not necessarily required for material utilization (Naujock & Blechschmidt, 2021).

The continued use of paper towels as single-use items in public institutions, catering and trade has contributed to residual waste in public institutions, with approximately 70,000 tons generated annually in Germany (Euromonitor International, 2017). Despite separate collection in washrooms, these towels are typically incinerated with other residual waste, dismantling their cellulose backbone and releasing carbon dioxide. In contrast to this practice, used paper towels represent a promising, homogeneous and relatively clean waste fraction for repurposing.

Conventional recycling processes, as applied to paper and cardboard, are only practicable to a limited extent for paper towels. In contrast to e. g. standard writing paper, paper towels contain wet strength agents, which significantly hinder the disintegration of the paper towels and consequently complicate the recycling process (Federal Environmental Agency, 2023). In addition, existing take-back and recycling schemes for used paper towels, as selectively offered by companies such as Tork and Kimberly-Clark, are currently implemented only on a limited scale (Kimberly-Clark; Tork). In contrast, upcycling offers a promising alternative. The cellulose fibers can be enzymatically hydrolyzed into glucose, a versatile substrate for biotechnological processes, thus enabling the production of valuable compounds, such as bioplastic precursors, biosurfactants and organic acids. This contributes to meeting the demand for renewable raw materials without imposing any additional restrictions on the public, thereby enhancing the acceptance of sustainable waste management practices.

No specific methods for the enzymatic saccharification of paper towel waste as a standalone substrate have been described to date. In contrast, numerous established approaches exist for the processing and saccharification of lignocellulosic raw materials such as wood or straw, which generally require a combination of mechanical, thermal, and chemical pretreatment steps to remove lignin and increase enzyme accessibility (Kaltschmitt et al., 2016).

A recent study investigated the enzymatic saccharifiability of mixtures of beech wood cellulose and municipal biowaste. Beech wood sawdust was fractionated into cellulose and non-cellulosic components using an Organosolv process, while the biowaste was sterilized at 100°C for several hours prior to saccharification. The resulting hydrolysates were evaluated for their suitability as a substrate for the cultivation of the mould *Mucor indicus* and the yeast *Saccharomyces cerevisiae*, as well as for their conversion to ethanol. This work, which follows a conceptually similar approach, underscores the potential of utilizing (ligno-)cellulosic residues and waste streams, particularly when considering regional feedstock availability (Rudnyckyj et al., 2024).

Another recent study examined the valorization of paper towel waste fibers through their conversion into rigid board materials for potential building applications. Various manufacturing approaches and adhesive systems, including starch- and casein-based formulations, were evaluated, and the resulting panels were characterized in terms of their mechanical, thermal insulation, and moisture-related properties. While the developed materials exhibited promising insulation performance and mould behavior comparable to conventional wood-based products, their mechanical strength was insufficient for load-bearing applications. These findings highlight both the potential and the limitations of paper towel waste as a resource, emphasizing the need for alternative utilization pathways (Payne, 2023).

A further study investigated the chemical depolymerization of paper towel waste, focusing on the effect of organic carbonates during microwave-assisted digestion. The study utilized paper towel waste sourced from Hong Kong International Airport. (Dutta et al., 2020)

Another previous study investigated the enzymatic saccharification of various waste paper materials using cellulases derived from *Trichoderma viride*. The authors evaluated sugar release across multiple incubation periods and observed that most substrates exhibited their highest sugar yields during the initial stages of hydrolysis. Among the tested materials, brown envelope paper, foolscap paper and office paper showed comparatively high sugar yields, whereas other materials displayed lower conversion efficiencies. (Mokatse & van Wyk, 2021) These results demonstrate the general feasibility of enzymatic cellulose hydrolysis from paper-based waste and underline the strong influence of substrate composition and structure on saccharification performance. However, paper towels as a distinct waste fraction were not considered in that study, and systematic investigations into their enzymatic saccharification behavior remain lacking.

Against this background, the present study aims to explore the enzymatic conversion potential of used paper towels into glucose and assesses its suitability for selected biotechnological applications by evaluating the growth of relevant prokaryotic and eukaryotic production microorganisms. Furthermore, this study includes the development of a sorting strategy for paper towels using a model municipality. Since the development of a collection concept is highly dependent on specific regional conditions, it is not considered in detail within the scope of this study and should be adapted accordingly to the local circumstances. In contrast, the sorting concept for washroom waste is analyzed in greater detail, as the composition of washroom waste is assumed to be relatively consistent across different regions.

2. MATERIALS AND METHODS

2.1 Collecting and sorting concept

To develop a concept for the collection, sorting and transport of washroom waste in an example city of medium size (approximately 41,500 inhabitants), a cadaster of public facilities was initially established and a survey was distributed to the majority of public facilities within the model municipality. The survey addressed the categorization of the public facilities, methods of hand-drying employed in washrooms, the number of individuals using the facilities, annual paper towel consumption and the types or brands of paper towels utilized. The data collected was used to estimate the amount of washroom waste generated and served as a database for the planning of the logistics for paper towel collection in collaboration with the local waste management organization.

By collecting and manually sorting samples of washroom waste from several public facilities, the percentage by weight as well as the types of impurities were determined. To extend the storability of the paper towels for further experiments, the paper towels were air dried. In order to minimize impurities prior to collection, a notice was displayed in the washrooms encouraging the separate disposal of paper towels.

For the optimization of waste sorting from all public facilities within the municipality, a trial sorting was performed at a pilot-scale sorting facility. This procedure included sieving into four fractions (< 10 mm, 10-30 mm, 30-80 mm, > 80 mm) and the use of a near-infrared sensor.

2.2 Saccharification of paper towels

2.2.1 Enzyme analysis

Commercial cellulases derived from the fungal species *Trichoderma reesei*, as well as commercially available cellulase blends, were investigated for the saccharification of unused reference paper towels at a shaking flask scale. For characterization and comparison of the enzymes, total cellulase activity (including endo-, exo- and β- glucosidases) was assessed using the dinitrosalicylic acid (DNS) assay, which quantifies the release of reducing sugars. The test principle is based on a color change reaction from yellow to brown, as reducing ends generated by enzymatic hydrolysis of the carboxymethylcellulose (CMC) substrate react with dinitrosalicylic acid (Miller, 1959, p. 427).

After sampling, the supernatant was obtained by centrifugation at 21,130 x g for 15 minutes, followed by immediate transfer of the supernatant. For each sample, 250 µL supernatant was mixed with 750 µL of a CMC solution (1.5% in 0.3 M $Na_3C_6H_5O_7$ buffer, pH 4.5) and incubated at 50°C for 20 minutes. Subsequently, 500 µL of DNS reagent (146.88 g/L $C_4H_4KNaO_6$ x H_2O, 4 g/L Na_2SO_4, 4 g/L phenol, 5.07 g/L 3,5-dinitrosalicylic acid, 10.19 g/L NaOH, brought up to 1 L with deionized water) was added. Each sample was boiled at 100°C for 5 minutes and immediately placed in an ice bath for 5 minutes. Photometric analysis was performed using a 96-well plate at a wavelength of 546 nm in a plate reader (Multiskan Go, ThermoFisher Scientific Inc.). For background absorption correction, a negative control was prepared for each sample by omitting the incubation step at 50°C according to test instructions. A positive control was established using a standardized dilution of commercial cellulases from *T. reesei* (C2730 Sigma-Aldrich).

2.2.2 Saccharification parameters and by-product analysis

The saccharification experiments of the paper towels were conducted at three different scales: 100 mL shaking flasks (50 mL working volume), a 15 L reactor (7 – 10 L working volume), and a 1000 L pilot scale reactor (800 L working volume). For both commercially available cellulase blends and cellulases from filamentous fungi, optimal parameters, including temperature and pH, were determined using shaking flask experiments conducted with an orbital shaker at 100 rpm. Additionally, the optimal enzyme-to-substrate ratio and saccharification duration were assessed. Optimization of substrate load (mass of substrate per liter) was subsequently transferred to larger scales.

The optimal cellulase blend for paper towel saccharification was identified by comparing saccharification yields achieved with each blend under optimized conditions. pH stability in shaking flask experiments was maintained using a citric acid-phosphate buffer (0.452 g/L $C_6H_8O_7$, 0.752 g/L Na_2HPO_4, pH 5.0) with paper towels cut into 1 x 1 cm pieces. In larger scale experiments, deionized water was used as the saccharification medium, with pH adjustments made using phosphoric acid and sodium hydroxide. For the 15 L scale, paper towels were cut into 2 x 2 cm pieces, while in the 1000 L scale, they were mechanically shredded and suspended in deionized water. For comparability, all experiments in the shaking flask and 15 L scales were performed with unused reference paper towels to determine optimal parameters. The saccharification of used paper towels was investigated at pilot scale. For the scale-up from 15 L scale to 1000 L scale, the focus was on optimizing process parameters to maximize the saccharification yield. The corresponding pilot trials were primarily conducted for process optimization rather than statistical validation. Therefore, each experimental condition was investigated only once (n = 1) also due to the limited number of pilot trials. An overview of the pilot process parameters is provided in Table 1. The first 1000 L pilot trial primarily served as a technical commissioning run for start-up and integration of the plant equipment (including interconnection of components, shredding technology for paper towels) and was not conducted under fully optimized process conditions. Consequently, the data obtained from this trial were not considered in the subsequent evaluation.

The solution of glucose hydrolyzed from paper towels was concentrated into a storage-stable glucose syrup (50-60% total sugars) using a falling film evaporator (70-90°C) as part of three pilot campaigns. The resulting syrup was utilized to investigate the suitability of hydrolyzed glucose as a component in fermentation media. In the third campaign, the hydrolyzed glucose solution was sterile filtered prior to concentration to remove any introduced contaminants. Pure cultures of selected contaminants

TABLE 1: Variated process parameters in 1000 L pilot scale.

Process parameters	First pilot scale	Second pilot scale	Third pilot scale
Substrate	Unused reference paper towels	Used reference paper towels	Mixture of different used paper towels
Temperature in °C	50	55	55
Enzyme-to-substrate ratio in % (w/w)	5.0	5.0	4.5
Substrate load in g/L	20	40	44

were identified for one hydrolysis by 16S rRNA gene sequencing using standard primers. This was performed by an external laboratory.

The saccharification yields of various unused and used paper towels were calculated based on the glucan content, as determined by acid total hydrolysis, but were adjusted to reflect that the theoretical maximum mass of releasable glucose exceeds the pure glucan mass due to the incorporation of water molecules when glycosidic bonds are cleaved during hydrolysis. Glucose quantification was carried out via high-performance liquid chromatography (HPLC). Potential by-products (e. g. acetic acid) that may form during saccharification were also analyzed via HPLC. The presence of metabolites resulting from possible contamination (ethanol, acetic acid and lactic acid) was also examined via HPLC. The HPLC system was equipped with an organic acid resin column (polystyrene-divinylbenzene copolymer, CS-Chromatographic Service GmbH, 300 x 8 mm) operated at 40°C. The mobile phase consisted of 0.004 M sulfuric acid and was delivered at flow rate of 0.8 mL/min under isocratic conditions. The injection volume was 10 µL, and the total run time was 30 min. Detection was performed using a refractive index detector. Sugars (glucose, cellobiose/sucrose, xylose/fructose) were quantified by HPLC using external standards for calibration. Sample concentrations were calculated from the corresponding calibration curves. Samples for HPLC analysis were centrifuged at 21,130 x g for 20 minutes and filtered through a 0.2 µm pore size membrane prior to measurement. The saccharification yield was calculated as described in Formula 1.

$$(\text{Released glucose [g]} \cdot 0.9) \,/\, \text{paper towel glucan content [g]} \cdot 100\% = \text{saccharification yield [\%]} \quad (1)$$

2.2.3 Sanitization

Since paper towels from washroom waste may be contaminated, particularly with fecal bacteria, spiking experiments using *Escherichia coli* (DSM 498) as an indicator organism were conducted to investigate under which sanitization conditions the fecal bacteria can be fully inactivated, thereby ensuring the hygienic safety of the waste.

For the spike experiments, an initial streak plate of *E. coli* was prepared on CCA agar (Sigma-Aldrich, CAS 1473387-52-8) for isolation of single colonies. Subsequently, a single colony was transferred into a 250 mL shaking flask containing 100 mL of medium composed of 5 g/L meat peptone and 3 g/L meat extract, and was cultivated at 36°C for 24 h. Afterwards, the saccharification setups (see Section 2.2.2) were spiked with 1 mL resulting *E. coli* culture. The incubation of the spike experiments was conducted in a water bath at 50°C and at 70°C. Samples were withdrawn after 0.5 h, 1 h, 2 h and 24 h. To evaluate the inactivation of *E. coli* in the spiked saccharification experiments, serial dilutions of 1 mL samples from each setup were prepared using 0.154 M NaCl. Subsequently, 0.1 mL aliquots were plated on agar consisting of 5 g/L meat peptone, 3 g/L meat extract and 12 g/L agar-agar. The efficacy of inactivation was assessed by enumerating *E. coli* colonies following incubation of the plates at 36°C for seven days.

2.3 Suitability of hydrolysis glucose in fermentation media

To investigate the suitability of glucose syrup derived from paper towel saccharification as a universal energy and growth substrate, various biotechnologically relevant microorganisms were cultivated. The following microorganisms were tested: *Saccharomyces cerevisiae* (baker's yeast) as an ethanol producer, *E. coli* (DSM 498) for a diverse product spectrum, *Lactobacillus rhamnosus* as a producer of a precursor of bioplastic (polylactic acid), *Cupriavidus necator* (DSM 545) for PHA-production and *Penicillium verruculosum* (neotype strain, DSM 2263) as a representative for filamentous fungi like *Aspergillus niger* for citric acid production. The growth of bacteria and *S. cerevisiae* was determined by measuring the optical density at a wavelength of 600 nm (OD600). For the filamentous fungus, growth was assessed by biomass production via dry matter (DM) measurement after drying to constant weight at 105°C. Outliers were identified using Grubbs' test (two-tailed, significance level α = 0.05) and removed in a single iteration. Statistical significance of differences between syrup-based media and the control was evaluated using Student's t-test (two-tailed, unpaired, significance level α = 0.05, assuming equal variances).

S. cerevisiae was cultivated in 100 mL shaking flasks (working volume 50 mL) containing 15 g/L glucose and 5 g/L peptone from casein at pH 7.4, at 36°C for 24 h. *E. coli* was grown in a medium consisting of 0.001 M $MgSO_4$, 0.0001 M $CaCl_2$, 0.001 M filter-sterilized Thiamine-HCl x 2 H_2O, 2 g/L glucose, 6 g/L Na_2HPO_4, 3 g/L KH_2PO_4, 1 g/L NH_4Cl, and 0.5 g/L NaCl, at a pH of 7.4, at 36°C for 24 h (German Collection of Microorganisms and Cell Cultures GmbH, 2022). The cultivation of *L. rhamnosus* was performed in a medium containing 10 g/L peptone from casein, 10 g/L meat extract, 5 g/L yeast extract, 20 g/L glucose, 1 g/L Tween-80, 2 g/L K_2HPO_4, 5 g/L sodium acetate, 2 g/L $(NH_4)_3$ citrate, 0.2 g/L $MgSO_4$ x 7 H_2O and 0.05 g/L $MnSO_4$ x H_2O, at a pH of 6.5, at 37°C for 48 h (German Collection of Microorganisms and Cell Cultures GmbH, 2007). *C. necator* was grown in a medium composed

of 10 g/L glucose, 4.3 g/L Na_2HPO_4, 1.5 g/L KH_2PO_4, 1 g/L $(NH_4)_2SO_4$, 0.2 g/L $MgSO_4$ x 7 H_2O, 0.01 g/L $CaCl_2$ x 2 H_2O, 0.06 g/L $Fe(NH_4)_2(SO_4)_2$ and 1 mL of a trace element solution consisting of 0.67 g/L $CoCl_2$ x 6 H_2O, 0.03 g/L $MnCl_2$ x 4 H_2O, 0.3 g/L H_3BO_3, 0.01 g/L $CuSO_4$ x 5 H_2O, 0.1 g/L $ZnSO_4$ x 7 H_2O and 0.0085 g/L $NiSO_4$ at a pH of 7.0 at 30°C for 72 h (Berezina, 2013, p. 193). *P. verruculosum* was cultivated in a medium containing 15 g/L glucose and 5 g/L peptone from casein with pH adjusted to 5.5, at 31°C for 144 h. All cultivations were performed using an orbital shaker. Agitation was maintained at 150 rpm for *C. necator* and at 100 rpm for all other cultures.

To assess the suitability of the glucose syrups derived from the pilot experiments for cultivation of the model organisms, the syrups were used as the main, but not necessarily the only, carbon source in place of laboratory-grade glucose. For comparability, the same cultivations were also conducted using identical media but with laboratory-grade glucose instead (control medium). Both the control medium and the media with the respective syrups were adjusted to the same initial glucose concentration. However, as a result of the varying viscosities of the syrups, it was difficult to accurately set the target glucose concentration. Due to the resulting deviations in glucose concentration between syrup-based media and controls, the increase in optical density or dry matter was normalized to the initial glucose concentration of each medium. For cultures grown in syrup-based media, the respective growth parameter was multiplied by glucose concentration of the control medium divided by glucose concentration of the syrup-based medium (factors ranging from 0.8595 to 1.3297). Glucose consumption during cultivation was verified by HPLC analysis, ensuring its utilization by the microorganisms for growth. Total sugar concentrations varied between media due to the presence of additional fermentable sugars in the syrup-based media and were not taken into account as the syrups were intended to substitute glucose rather than the entire carbon source. Non-glucose sugars were considered co-components, with glucose defined as the target substrate. Therefore, no negative control without glucose or syrups was included.

3. RESULTS AND DISCUSSION

3.1 Collection and sorting process

According to the conducted survey, paper towels were identified as the predominant drying method in washrooms across most public facilities (90%) within the model municipality. A diverse range of paper towel brands and types was reported, reflecting variability in supplier selection among facilities. Within the model region, an estimated 71.2 tons of dry paper towels were used annually by approximately 41,500 inhabitants. This corresponds to a total washroom waste generation of 87.4 tons, of which 83.8 tons consist of moist used paper towels. Following sorting, 80.2 tons of moist used paper towels can be made available for saccharification across the entire model municipality. The paper towel waste used in this study consisted of samples totaling more than 150 kg.

Manual sorting of the collected washroom waste samples from volunteering public facilities indicated impurity levels ranging from 0.12% to 29.38% by weight. Notably, in almost one-third of these facilities, impurity levels below 1% (w/w) were observed, while approximately 58% exhibited impurity levels up to 12% (w/w) and approximately 8% showed impurity levels exceeding 12% (w/w). The implementation of washroom notices encouraging proper disposal practices appeared to have a positive effect on impurity content, resulting in a decrease from 2.17% (w/w) to 0.86% (w/w) in one participating facility. Common impurities included tissues, plastic packaging, cardboard rolls, and paper products (Figure 1).

In the pilot-scale sorting trials, sieving eliminated 0.33% (w/w) of the whole washroom waste. This fraction mainly consists of small impurities and small paper towel pieces. With the employed two-step near-infrared sorting, a paper towel loss of 4.3% (w/w) was observed, while the sorted fraction reached a purity of 99.6% paper towels by weight. A total of 91.0% (w/w) of existing impurities was eliminated from the sorted washroom waste. Despite the use of screening and near-infrared technology during trial sorting, additional measures such as wind sifting were identified as necessary to further minimize impurities. This was particularly important for eliminating rolling impurities, such as bottles, which tend to roll on the conveyor belt and thereby impede the sorting process. The required mechanical sorting can be effectively managed with existing technologies.

3.2 Saccharification of paper towels

The investigation of various enzyme blends revealed that the highest saccharification yield (98.4%) was

FIGURE 1: Manual sorting of a washroom waste sample from a participating facility into the target fraction of paper towels and the impurity fraction: a) unsorted washroom waste, b) and c) impurity fraction, d) target fraction of paper towels (laid out for drying).

achieved with one commercially available enzyme blend Novonesis Cellic® CTec3 HS under optimal process conditions with unused reference paper towels as substrate in shaking flask scale. Yields of up to 88.3% were attained by other commercially available enzyme blends. Although total cellulase activity was maintained constant across all assays, commercially available preparations derived from individual filamentous fungi generally exhibited lower efficiencies; for instance, saccharification yields of 46.4% and 70.8% were achieved with cellulase preparations from *Trichoderma reesei from* various suppliers/manufacturers. Furthermore, greater batch stability and broader pH tolerance were displayed by Cellic® CTec3 HS, representing significant advantages for industrial applications. All assays were analyzed after 48 h of saccharification.

In shake flask experiments, the optimal parameters for saccharification with Cellic® CTec3 HS were determined to be 55°C, pH 5.0, 100 rpm, 20 g/L substrate load, 5% (w/w) enzyme-to-substrate ratio and 48 h incubation time. Under these conditions, a maximum saccharification yield of 98.4 ± 1.5% (n = 3) was achieved with unused reference paper towels. Additionally, saccharification across 12 different paper towel brands was tested. Even the paper towels with the lowest saccharification yields reached 55.4% (n = 2) conversion rates under the aforementioned conditions.

As shown in Figure 2, on a 15 L scale, a saccharification yield of 63.3 ± 2.8% (n = 3) after 48 h and 56.9 ± 1.5% (n = 3) after 24 h was achieved under almost identical conditions except for stirring at 200 rpm and an increase of the substrate load to 40 g/L.

This reduction in saccharification yield from 98.4% in shaking flasks to 63.3% at the 15 L scale after 48 h is primarily attributed to altered mixing dynamics and contamination by environmental microorganisms, rather than to the increase in substrate load. This assumption is supported by the observation that the substrate load could be increased to 40 g/L at the 15 L scale with only a slight reduction in saccharification yield of 3-5 percentage points.

For unused reference paper towels, the application of an additional sanitization step at 90°C prior to saccharification (for one or two hours) to combat microbial contamination resulted in only a slight increase in saccharification yield to 62.6 ± 2.4% (n = 4) after 24 h (in case of sanitization for two hours), which does not justify the substantial increase in energy expenditure. Especially considering that spike experiments demonstrated that prolonged treatment at 50 and 55°C during saccharification as well as short-term pretreatment at 70°C for 30 minutes were sufficient for complete inactivation of *E. coli*. Thus, an additional sanitization step was considered unnecessary from a purely hygienic perspective. However, since the growth of environmental microorganisms (particularly spore-forming species) was observed even with unused paper towels, and because a high microbial load can result in sugar depletion due to metabolization, this issue is expected to be even more pronounced with used paper towels, which exhibit a higher level of contamination. For this reason, sanitization was implemented at the pilot-scale for used paper towels despite the substantial energy requirements. Nonetheless, sanitization alone did not prove sufficient to completely prevent microbial growth during saccharification. Consequently, it was deemed necessary to reduce the saccharification duration from the optimal 48 to 24 h. The increase in sugar yield that could be obtained by extending saccharification for an additional 24 h was not sufficient to justify the additional energy required to maintain a temperature of 55°C during this period.

According to the data shown in Figure 3, after 24 h a saccharification yield of 58.6% was achieved in a 1000 L pilot-scale experiment with used reference paper towels

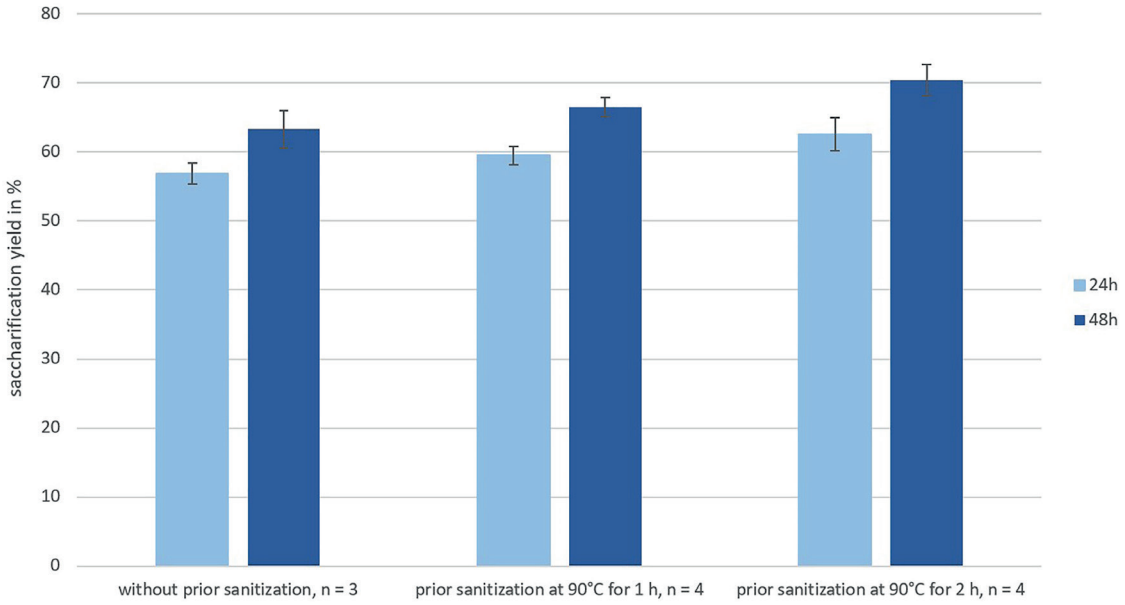

FIGURE 2: Effect of sanitization time at 90°C on the saccharification yield of unused reference paper towels after 24 h and 48 h (15 L scale). Conditions: 55°C, pH 5.0, enzyme-to-substrate ratio 5% (w/w), substrate load 40 g/L; yield expressed relative to glucan content.

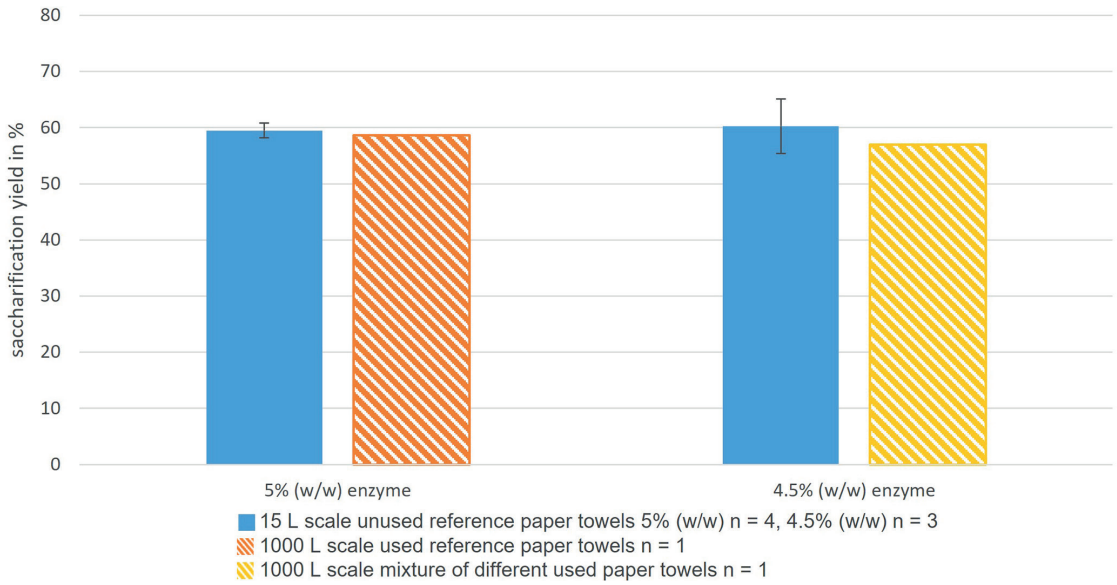

FIGURE 3: Saccharification yields of used and unused reference paper towels after 24 h of enzymatic hydrolysis at the 15 L and 1000 L scales (55°C, pH 5.0; 4.5% and 5% enzyme-to-substrate ratio; 40 and 44 g/L substrate load; prior sanitization at 90°C for 1 h).

as substrate (at 55°C, pH 5.0, 60 rpm, 40 g/L substrate load and an enzyme-to-substrate ratio of 5% (w/w)). Compared to the saccharification yield obtained at the 15 L scale (59.5 ± 1.3%, n = 4), no notable scale-up effect was observed. With a slightly elevated substrate load of 44 g/L and mixed-type used paper towels, a comparable saccharification yield could be achieved (57%), even when the enzyme-to-substrate ratio was simultaneously reduced to 4.5% (w/w). As shown in Figure 3, it was also confirmed at the 15 L scale that this 10% reduction in enzyme-to-substrate ratio did not affect the saccharification yield (60.3 ± 4.8%, n = 3).

Even after concentration of the glucose solution to a total sugar content of 50-60%, spore-forming environmental microorganisms could still be detected in the glucose syrup, indicating their survival under these conditions. However, no proliferation of these microorganisms was detected during storage of the syrups obtained from pilot-scale experiments 1 and 2. Proliferation was only observed after dilution to lower sugar concentrations. In order to further ensure the purity and stability of the product, the hydrolysate from the third pilot-scale experiment was subjected to sterile filtration before concentration. In total, three glucose syrups were produced during the pilot-scale experiments.

3.3 Suitability of hydrolysis glucose in fermentation media

To evaluate the suitability of the syrups, each microbial test organism was cultivated in media in which hydrolysis-derived sugars, primarily, but not exclusively, glucose (syrups 1-3), served as the main carbon source, and the results were compared to growth in control media containing laboratory-grade glucose. Figure 4 presents the final optical density or, for *P. verruculosum*, the dry biomass achieved under these conditions. The data was normalized to the corresponding value obtained with media containing laboratory-grade glucose, which was set at 100%. For each organism, six biological replicates were performed.

Saccharomyces cerevisiae showed equal or slightly enhanced growth in all syrup-based media compared to the control. The strongest effect was achieved with syrup 1, resulting in 126 ± 5% of the control, which was statistically significant. Growth supported by syrup 2 and syrup 3 was close to that obtained with the control medium (111 ± 12% and 100 ± 9%, respectively), as the differences were not statistically significant. Variability across biological replicates remained relatively low, indicating robust utilization of the glucose syrup by the yeast. It should be noted, however, that the tested syrups contained additional sugars such as xylose/fructose and cellobiose/sucrose (Table 2), which were absent from the control medium. Since hemicellulose that is also present in paper towels is a polysaccharide composed of various hexoses and pentoses (Shinde et al., 2022, p. 2), and *S. cerevisiae* is capable of metabolizing sugars other than glucose (e. g. fructose and sucrose (Kaltschmitt et al., 2016, p. 1503)), the slight increase in growth in syrup-based media is likely due to the overall higher concentration of fermentable sugars. Nevertheless, the results clearly demonstrate that the hydrolysis-derived glucose syrups are well suited as main carbon sources for *S. cerevisiae*, as none of the three syrups exhibited any evidence of inhibitory components relevant for this yeast.

Escherichia coli exhibited a slightly different trend. Growth in syrup 1 and 3 (115 ± 4% and 113 ± 11%, respectively) significantly exceeded that in the control, while syrup 2 showed significantly reduced growth (79 ± 6%). Variability was low for syrups 1 and 2 but increased with syrup 3. These results suggest that *E. coli* can metabolize the hydrolysate sugars efficiently, although inhibitory impurities may be present in syrup 2, limiting performance.

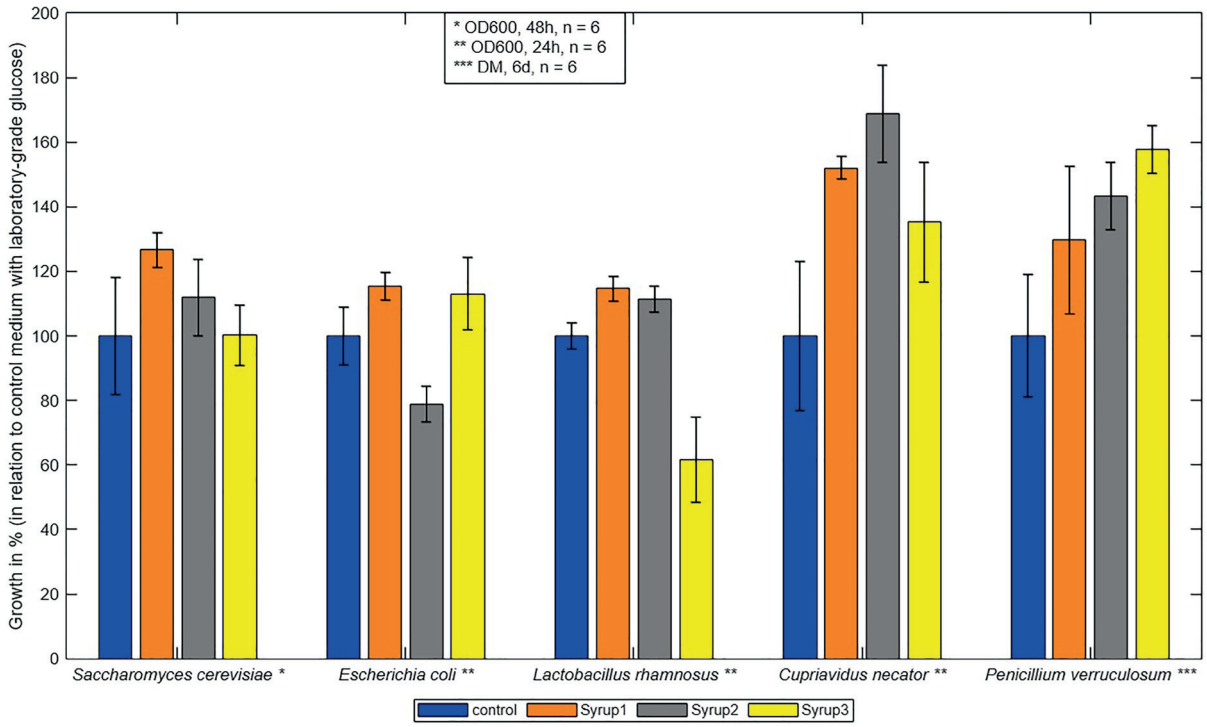

FIGURE 4: . Relative growth of five microbial species cultivated with three syrup-based media compared to laboratory-grade glucose-based control media. Growth of *Saccharomyces cerevisiae* (48 h, OD600), *Escherichia coli* (24 h, OD600), *Lactobacillus rhamnosus* (24 h, OD600), *Cupriavidus necator* (24 h, OD600) and *Penicillium verruculosum* (6 days, dry matter). Values are expressed as percentage of the control medium (100%). Bars represent mean ± standard deviation (n = 6).

One inhibiting compound might be lactic acid that was found in the hydrolysis syrups and might stem from microbial contamination of the paper towels. Sequence analysis of contaminants revealed the presence of, among others, *Bacillus licheniformis*, *Paenibacillus thailandensis*, and *Bacillus smithii*, which are able to survive under the conditions prevailing during pretreatment and hydrolysis and are, in principle, capable of lactic acid formation (Chawla & Goyal, 2024, pp. 2-3; Khianngam et al., 2009, pp. 565–566; Zhao et al., 2024, 1-4). Lactic acid is known to have antimicrobial properties (Alakomi et al., 2000, p. 2001). However, these are mainly attributed to its undissociated form at low pH-values (Gonçalves et al., 1997, p. 350), whereas in the media used with *E. coli* the pH was adjusted to 7.4. Inhibitors could also originate from additives or wet-strength agents used during paper towel manufacturing. However, the chemical characterization and quantification of these additives was not included in this study and is not feasible given the variable composition of paper towel waste in terms of both the types and proportions of different components. Furthermore, the composition and number as well as the amount of additives in mixtures of collected paper towels from real-world implementation are expected to vary considerably, since the proportions of paper towels from different manufacturers or brands can differ between collection batches. Since syrup 1 and 2 were derived from the same brand and type of paper towels, it is unlikely that manufacturing residues were the cause of the inhibited performance from syrup 2 compared to syrup 1. Moreover, the paper towels utilized for the hydrolysis leading to syrup 2 and 3 were used and therefore a similar level of contaminants was present for both.

Growth of *Lactobacillus rhamnosus* was significantly enhanced in media containing syrup 1 and syrup 2 (115 ± 4% and 111 ± 4%, respectively), whereas growth was significantly reduced with syrup 3 (62 ± 13%), accompanied by increased variance among biological replicates. These findings suggest that the composition of syrup 3 may negatively affect this lactic acid bacterium for one of the three potential reasons: due to inhibitory substances present in the various types of paper towels or contamination of the individual substrate sample tested or

TABLE 2: Sugar concentrations and density of the three hydrolysis syrups.

	First pilot scale	Second pilot scale	Third pilot scale
Glucose concentration in g/L	378.1	466.8	532.7
Cellobiose/sucrose concentration in g/L	23.8	18.2	26.7
Xylose/fructose concentration in g/L	100.9	111.2	158.8
Density in g/cm³	1.21	1.26	1.34

the presence of the aforementioned lactic acid. However, at higher pH values, inhibition of *L. rhamnosus* is mainly caused by intracellularly accumulated lactate, rather than by extracellular lactic acid (Gonçalves et al., 1997, p. 350) as it is the undissociated form of lactic acid at low pH values that can penetrate cytoplasmic membranes (Alakomi et al., 2000, p. 2001). In this instance, an initial pH-related effect of lactic acid on *L. rhamnosus* can be ruled out, since all media were adjusted to a neutral pH. Compared to the other microorganisms tested a higher glucose concentration is necessary for *L. rhamnosus*. For this the syrups were diluted less than in the other media. It is therefore possible that *L. rhamnosus* is not necessarily more susceptible to the accompanying substances than the other microorganisms tested, but rather that the higher concentration of such compounds was the decisive factor. It should be noted that *L. rhamnosus* is only one representative for lactic acid bacteria. Therefore, no general conclusions regarding the growth of lactic acid bacteria on the syrup can be drawn from these results alone. As different representatives of this group of production microorganisms may vary in their sensitivity, it is generally essential to carry out suitability tests using the relevant strains.

In contrast, *Cupriavidus necator* exhibited statistically significantly improved growth with all syrups. Syrup 2 supported the highest increase (167 ± 15%), followed by syrup 1 (152 ± 4%) and syrup 3 (135 ± 19%). Since *C. necator* is known for its highly versatile metabolism (Le Zhang et al., 2022, p. 2), the aforementioned additional sugars present in the syrups (e.g. cellobiose/sucrose and xylose/fructose, and possibly other mono- and oligosaccharides) may contribute to the enhanced growth. Moreover, this pronounced stimulation suggests that the hydrolysis-derived syrups may also contain other growth-promoting compounds, possibly organic acids, as indicated by the detected lactic acid.

Penicillium verruculosum also showed enhanced growth and reached 129 ± 23% in syrup 1, 143 ± 10% in syrup 2 and 157 ± 7% in syrup 3, with growth in syrups 2 and 3 statistically significantly higher than in the control. The partially wide error margins observed may be a result of limitations in the robustness of biomass quantification at the small culture scale employed in this study. Based on the results, it can be assumed that no inhibitory compounds relevant to *P. verruculosum* are present in the syrups. On the contrary, the data suggest that the syrups may contain favorable accompanying compounds, e.g. additional sugars from hemicellulose or inactivated enzymes from the saccharification process, as *P. verruculosum* is able to metabolize not only glucose but a wide range of carbohydrates, organic acids, fatty acids, amino acids (Rabha & Jha, 2017).

4. CONCLUSIONS

This study demonstrates the suitability of repurposing used paper towels as a sustainable and efficient source of glucose for biotechnological applications. The development of a suitable sorting concept, consisting of sieving, wind sifting and two-step near-infrared sorting, enabled a nearly pure paper towel feedstock for efficient enzymatic saccharification. Using Novonesis Cellic® CTec3 HS resulted in over 98% saccharification yields under laboratory conditions and satisfactory and reproducible conversion rates at both 15 L and 1000 L scales in stirring reactors (63.6% and 58.6% after 24 h with used reference towels), despite challenges such as mixing dynamics and environmental microbial contamination. While a decrease in saccharification yield was observed when scaling up from laboratory to 15 L scale, no further scale-up effects were detected between the 15 L and 1000 L scales, suggesting that the process can be scaled up further in stirring reactors. The slight difference in saccharification yield at the 15 L scale compared to the 1000 L scale can most likely be attributed to the change from unused reference paper towels in the 15 L scale to a mixture of different used paper towels (with a different composition compared to the reference towels) in the 1000 L scale. Additionally, it was demonstrated that the increase in substrate load to 44 g/L and reduction in enzyme-to-substrate ratio by 10% to 4.5% (w/w) can be achieved without significant losses in saccharification yield.

While *E. coli* as a fecal indicator can be completely inactivated during saccharification, the presence of spore-forming environmental microorganisms necessitates either a pretreatment of the paper towel waste or a posttreatment of the hydrolysate. However, a one-hour sanitization step at 90°C prior to saccharification alone was insufficient to control the contaminants. Given the need for additional contamination control measures, and considering the only marginal increase in saccharification yield observed with extended saccharification times, a process duration of 24 h was selected as optimal over 48 h, balancing energy consumption, process efficiency and microbial stability. In the third pilot-scale experiment, sterile filtration of the hydrolysate was implemented to completely eliminate any remaining contaminants in the product. Results from the 15 L scale with unused reference paper towels encourage the assumption that, when sterile filtration is employed, the initial sanitization step prior to processing can be omitted. However, this assumption has not been tested with used paper towels. Thus, further investigation is required.

The optimal parameters for saccharification of used paper towels at scales ≥ 1000 L were determined to be as follows: 1 h sanitization at 90°C, substrate load of ≥ 44 g/L, pH 5.0, 60 rpm, enzyme-to-substrate ratio of 4.5% (w/w), temperature of 55°C and a saccharification duration of 24 h.

In the growth experiments, the hydrolysis-derived glucose syrups (50-60% total sugar content) generally supported equal or improved growth compared to laboratory-grade glucose for most of the tested microorganisms, indicating good overall suitability as main carbon sources. In case of the metabolically versatile organisms, like *S. cerevisiae*, *C. necator* and *P. verruculosum*, that can utilize sugars other than glucose, enhanced growth might be due to additional fermentable sugars and other potentially growth-promoting co-components present in the syrups. Negative effects were limited to individual syrup-organism combinations and are most likely related to the presence

of inhibitory impurities and potentially to concentration effects arising from different dilution requirements, rather than to a fundamental incompatibility of the syrups. Overall, the syrups performed well in this growth analysis, but their variable compositions and organism-specific sensitivities underline the need for strain- and batch-specific suitability testing.

In summary, the findings highlight the potential of paper towel waste in local bioeconomy value chains, contributing to circular material flows and the generation of value-added biotechnological products. This approach offers a practical pathway for carbon upcycling, combining environmental benefits with high biotechnological potential that can be implemented with existing technologies.

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