DESIGN FOR TRANSFORMATION

ROTTERDAM

May 2024

Textile Waste Transformation
Pilot Project

BIOMIMICRY INSTITUTE

CIRCLE ECONOMY

We are a global impact organisation with an international team of passionate experts based in Amsterdam. We empower businesses, cities and nations with practical and scalable solutions to put the circular economy into action. Our vision is an economic system that ensures the planet and all people can thrive. To avoid climate breakdown, our goal is to double global circularity by 2032.

DESIGN FOR TRANSFORMATION

This initiative uses biomimetic system principles to demonstrate the sequential breakdown of apparel into new nutrient cycles by converting textile waste into novel, marketable feedstock for biocompatible materials within a specific region. Its many years of research, network development, and technical expertise underpin the implementation of regional pilots, as the one described in this report.

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EXECUTIVE SUMMARY

The goal of the Design for Transformation (D4T) pilot was to demonstrate a system of technologies capable of processing mixed textile waste, while generating biomimetic outputs based on textile waste streams arising in Rotterdam, the Netherlands.

The design of the pilot was based on several factors, namely: the ability to process unsorted textile waste, a sufficient technology readiness level, potential for scalability, and regional partners who are willing to collaborate in the short- and potentially long-term. The technology partners engaged in the pilot were as follows: Erdotex, BioFashionTech (BFT), EV Biotech (EVB), and the Netherlands Organisation for Applied Scientific Research (TNO).

BioFashionTech converted the cellulosic component of the mixed textile sample into glucose through enzymatic hydrolysis. The glucose component was then transferred to EV Biotech for use as a feedstock in bacterial fermentation of a class of biodegradable polyesters, polyhydroxyalkanoates (PHAs). The synthetic fibres not converted into glucose(polyester, nylon and polyurethane fibres) and their associated chemistries (colourants and finishes)—were dried and sent to TNO to undergo gasification to produce syngas (a mixture of hydrogen, carbon monoxide, carbon dioxide, methane, ethylene, including a small amount of aromatics and other elemental gases such as nitrogen.

Glucose was successfully produced from the textile waste with a concentration in the range of 40 - 63 grams per litre. The hydrolysis process was limited by various elements of the experimental design, such as the presence of non-target fractions or the formation of cello-oligosaccharides, which can be overcome in future iterations through various process improvements.

The textile-derived glucose was not only found to be comparable to commercial grade glucose in performance, but in some cases outperformed it. This indicates that the cellulosic fraction of a mixed textile waste might be a good source of carbon to produce glucose for PHA production. If this process can be scaled and is found to be economically feasible, it could be a good alternative to glucose derived from food crop starches.

The residual stream from the enzymatic hydrolysis step contained primarily synthetic fibres, such as polyester and nylon, as well as some remaining cellulose due to incomplete hydrolysis. This stream was successfully gasified into syngas at a conversion efficiency exceeding 70%wt. Additionally, samples containing 100% synthetic fibres were compared with samples containing the residual cellulosic fibres. Results indicate that the samples containing residual cellulosic fibres produced more hydrogen gas and had reduced amounts of tars and benzene.

REPORTING FORMAT

The Circle Economy team, in collaboration with technology partners, have compiled the pilot's experimental setup and results, as detailed in the following sections. In support of the future scaled success of the chosen pilot technology pathway, the Design for Transformation (D4T) technical team has included an analysis for each processing step, noting our proof-of-concept phase limitations and outstanding questions, as seen in the "D4T Outcomes Analysis" sections.

1. INTRODUCTION

1.1 PROBLEM DEFINITION

The fashion industry produces more than 110 million tonnes of fibre annually,[1] with a similar amount collectively discarded each year. While efforts to chemically recycle textiles are growing, they mostly focus on cotton, polyester, and their blends—but not other common fibres or blends, much less composite materials. Municipal solid waste providers are faced with the challenge of finding a suitable destination for unsorted and multi-material textiles—often resulting in incineration. The incineration of textiles is a great contributor to greenhouse gas (GHG) emissions and thus, climate change. As governments and citizens begin to turn away from both incineration and waste-to-energy due to the GHG emissions and other pollutants they cause, ban second-hand clothing imports to their countries, or veto new landfills, there are fewer and fewer options to dispose of post-consumer textiles.

1.2 DESIGN FOR TRANSFORMATION'S PILOT GOALS

Not all clothing can be recycled or reused, primarily due to items in poor condition and/or with complex compositions. This Design for Transformation (D4T) initiative takes the lowest value textile waste[2] from the sorting stream to prevent additional harm to the environment. Our solution aims to work towards developing a system that: (1) handles mixed materials; (2) preserves materials' highest value; (3) leverages existing infrastructure in order to scale; (4) provides end-of-life solutions for extended producer responsibility (EPR) strategies; (5) remediates toxic textile waste products; (6) reduces industry reliance on non-renewable raw materials; (7) identifies new methods for biomaterials manufacturing; (8) supports green job creation in the move towards a bioeconomy; and (9) supports biodiversity through pollution avoidance and alternative feedstock creation.

[1] Ütebay, B., Çelik, P., & Çay, A. (2020). Textile wastes: Status and Perspectives. *Waste in Textile and Leather Sectors*. doi:10.5772/intechopen.92234

[2] Clothing in the worst condition (for example, damaged or stained) that cannot be reused, or that is unable to be recycled either economically or technologically (for example, blended fibres)

D4T's work is inspired by nature, a system that solves problems through well-adapted designs, life-friendly chemistry, and efficient and effective material and energy use. Just as food is broken down in our digestive system through a multi-step process, we are linking together decomposition technologies to show a viable alternative to incineration, while helping next-generation innovators make non-toxic, non-fossil carbon-based new materials that will safely degrade or are innocuous (referred to from now onwards as 'biocompatible outputs'). D4T is developing new material flows and expanding what circularity means, looking less like one-size-fits-all technical loops and more like adaptable multi-nodal networks that operate locally.

The goal of this pilot was to demonstrate two to three technology pathways[3] that can successfully process mixed textile waste to create more biocompatible outputs based on textile waste stream compositions arising in Rotterdam, the Netherlands.

1.3 PROJECT APPROACH AND PARTNERS

The D4T global initiative is designed and managed by the D4T team in association with the Biomimicry Institute (TBI), and is collectively funded by multiple partners, including the Laudes Foundation, VF Foundation, Decathlon, and Stichting DOEN. D4T commissioned Circle Economy (CE) to act as a backbone organisation for a proof-of-concept pilot in the Rotterdam region, with other regional pilots concurrently conducted in Accra, Ghana and in Berlin, Germany. With input from D4T, CE established a Rotterdam Steering Committee uniting key regional stakeholders, recruiting relevant technology providers in the region, and coordinating and implementing the pilot. The City of Rotterdam was the main funding partner for the implementation of the Rotterdam proof-of-concept.

In September 2023 the Rotterdam Steering Committee was established, which convened key regional stakeholders: the City of Rotterdam (both the economic and circular economy departments), the Province of South Holland, the Port of Rotterdam, and the InnovationQuarter. In October 2023, the Steering Committee was consulted in the identification of regionally-relevant transformation pathways (i.e. combinations of industrially symbiotic technologies) for the decomposition of textiles. In December 2023, they advised on the selection of the most relevant pathway and the preliminary design of the Proof-of-Concept feasibility study. The Steering Committee also provided feedback on the final results of the project and the proposed next steps to continue the work in subsequent phases.

[3] A technology pathway refers to two or more technologies creating a symbiotic network.

2. EXPERIMENTAL SETUP & RESULTS

2.1 SYSTEM OVERVIEW

The design of the pilot was based on the following criteria: the ability to process unsorted textile waste, a sufficient technology readiness level,[4] potential for scalability, and regional partners who are willing to collaborate in the short- and potentially long-term.

The partners engaged in the pilot were as follows: Erdotex, BioFashionTech, EV Biotech and TNO. The following paragraphs describe the activities conducted by each of the selected technology partners, as well as textile waste providers.

Unsorted, mixed textiles from the lowest-value textile waste fractions were provided by the local collection and sorting partner, Erdotex. Erdotex was keen to engage in the pilot as this waste represents a cost centre – i.e. they cannot sell it so it is destined for incineration or for low-value reuse. The project team then removed hard parts and trims from a sample of these textiles, and cut them into approximately 1 by 1 centimetre pieces.

Utilising resources at Groningen University and analysis service of HAN_BioCentre, BioFashionTech (BFT) converted the cellulosic component of the textile sample into glucose. They achieved this transformation by initially shredding the sample into even smaller pieces, followed by enzymatic hydrolysis using BioFashionTech's unique enzyme blend tailored to target the cellulosic component. The cellulosic component within the sample was subsequently converted into glucose and recovered, while the residual fraction (mostly containing synthetic fibres) was separated and dried for thermochemical processing by TNO. BFT saw value in joining the pilot project since it allowed them to connect to a bigger system with other stakeholders whose knowledge and resources could help them scale their technology further in subsequent phases.

The glucose component was then transferred to EV Biotech (EVB) for use as a feedstock in bacterial fermentation to produce a class of biodegradable polyesters called polyhydroxyalkanoates (PHAs). EV Biotech had no previous experience producing PHAs from textile waste, so they were keen to experiment with new feedstocks.

The synthetic fibres not converted into glucose—(polyester, nylon and polyurethane fibres) and their associated chemistries (colourants and finishes)—were dried and sent to TNO to undergo gasification[5] to produce syngas

[4] "Technology Readiness Level" (TRL) 4 or above
[5] Gasification has been found to be a suitable alternative to incineration with energy recovery due to its lower carbon footprint (source), as well as its ability to break down harmful textile chemistries (source) and provide feedstock for a next generation of materials.

(mixture of hydrogen, and carbon monoxide, carbon dioxide, methane, and ethylene, including a small amount of aromatics and other elemental gases such as nitrogen.[6]

The intention for future iterations of the project is to transform this gaseous mixture into either methanol or methane, and use it as feedstock for the biotech industry to produce biocompatible outputs. In 2023, TNO conducted similar gasification tests on textile waste. They saw D4T as an opportunity to build upon this previous work using mixed textile waste. Their vision to create a growing body of work around textile waste aligns with D4T as they aim to focus on the most complex and/or lowest-value textile waste fractions, and have a strong focus on connecting with regional partners to scale new technology and processes as soon as possible.

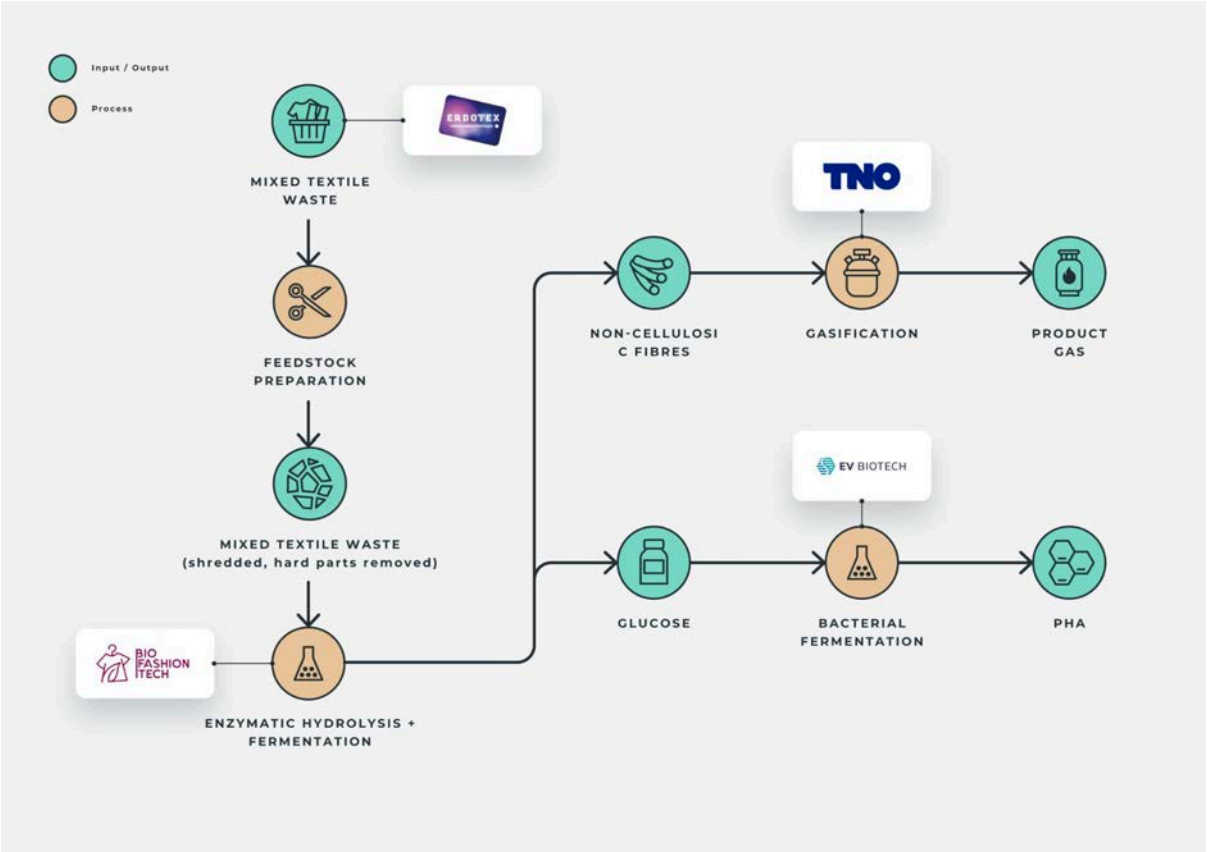


Figure 1. Visual representation of the end-to-end proof-of-concept pilot schematic

[6] This is an alternative to chemically recycling these plastic fibres, which is challenging for mixed textile waste and doesn't promote the shift to producing biocompatible materials.

2.2 TEXTILE WASTE

A sample of Erdotex's lowest-value textile fraction—which is left with no market after their conventional sorting process—was collected. From this sample, ten items of varying clothing types and materials were selected (see *Figures 2* and *3*). Hard parts (such as zippers and buttons) were removed and each item was cut into 1 by 1 centimetre pieces (*Figure 4*). The sample weighed a total of 2.4 kilograms. *Table 5* displays a breakdown of the material composition (based on the clothing labels).[7]

Figure 2. Sample of clothing items selected.

Figure 3. Sample of composite materials, or layered fabrics joined by adhesives, and textile with 'plastic' decorations.

[7] It should be noted that clothing labels are not always accurate at specifying material composition. For more information, refer to: Circle Economy. (2020). *Clothing labels: accurate or not?* Retrieved from: Circle Economy website

Figure 4. Sample of cuttings from the selected clothing items, cut into 1x1 cm pieces.

Item #	Product Type	Weight (g)	Polyester (%)	Cotton (%)	Polyurethane (%)	Nylon (%)
1	Shirt	58		100		
2	Jacket	442	100			
3	Tights	42	95		5	
4	Jacket	160	40	60		
5	Trousers	119	100			
6	Trousers	187	71		4	25
7	Jacket	304	34	66		
8	Sweater	239		100		
9	Jacket	532	67	33		
10	Sweater	300		100		
Total		**2383**				

Table 5. Material composition of the ten selected clothing items, provided by Erdotex, comprising the basis of the pilot feedstock.

2.3 ENZYMATIC HYDROLYSIS

The prepared textile waste was then sent to BFT to undergo enzymatic hydrolysis. For this processing step, we set out to answer the following research question:

- Can a mixed textile waste stream be effectively converted into glucose?
- Is the glucose of a quality that can be further processed into PHA?

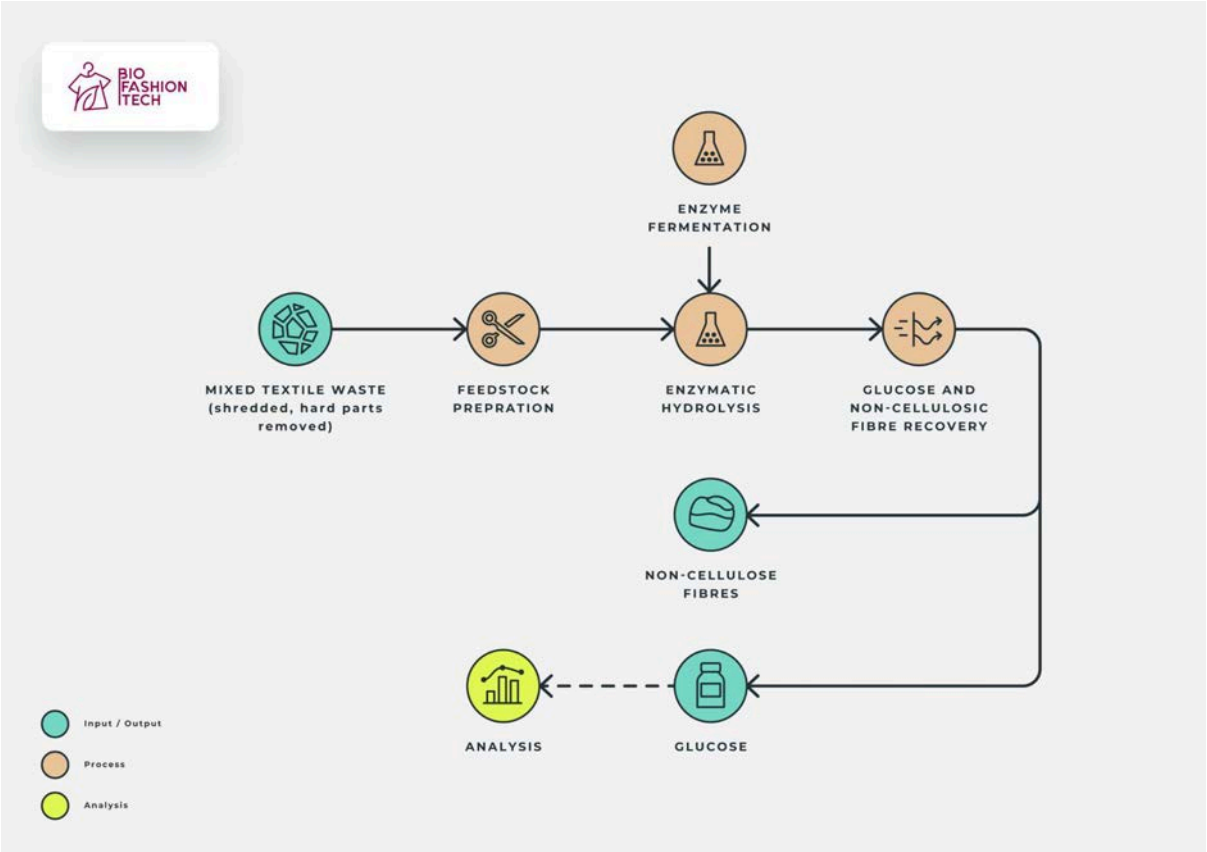


Figure 6. Schematic diagram of the process steps taken by BioFashionTech.

Feedstock preparation

BFT received a prepared sample weighing 2.38 kilograms and added 0.15 kilograms of additional items to compensate for the low volume of common product classes, such as jeans or t-shirts, which are rich in cellulosic polymers such as cotton and viscose. From the available 2.53 kilograms of available feedstock, 1 kg is shredded for further processing. The resulting composition, shown in *Table 7*, was deemed by Circle Economy to be a sufficiently representative composition for the target fraction of textile waste based on previous research.[8]

[8] Fashion for Good & Circle Economy. (2022). *Sorting for Circularity Europe*. Retrieved from: Fashion for Good report

Material	Weight (grams)	Share (%)
Polyester	1290	50.9
Cotton	1165	46.0
Elastane	14	0.6
Nylon	47	1.8
Viscose	16.5	0.7
Total	**2533**	**100**

Table 7. Composition of final feedstock from which the processed sample is taken

The fibres were reduced to the optimal size for the transformation processes through mechanical milling: from 0.5 to 3 millimetres in length.[9] The decision to limit the fibre size was based on two factors: (i) smaller fibres increase surface area, thereby making the transformation process more efficient by facilitating maximal enzyme access to cellulose; and (ii) the need to separate layered textiles (i.e. composites) that have been bonded together with adhesives.

In an effort to minimise the environmental footprint of the transformation process, additional pretreatment steps aimed at 'opening up' cotton fibres to enhance enzyme accessibility were omitted. While methods such as steam explosion, alkaline pretreatment, and sonication exist to achieve this objective, we excluded them to streamline the process.[10] Further analysis is required to weigh the potential benefit of implementing pretreatment processes.

Figure 8. Textile cuttings being ground into smaller pieces.

[9] Gritsch, S. M., Mihalyi, S., Bartl, A., Ipsmiller, W., Jenull-Halver, U., Putz, R. F., ... Guebitz, G. M. (2023). Closing the cycle: Enzymatic recovery of high purity glucose and polyester from textile blends. *Resources, Conservation and Recycling*, *188*, 106701. doi:10.1016/j.resconrec.2022.106701

[10] Gritsch, S. M., Mihalyi, S., Bartl, A., Ipsmiller, W., Jenull-Halver, U., Putz, R. F., ... Guebitz, G. M. (2023). Closing the cycle: Enzymatic recovery of high purity glucose and polyester from textile blends. *Resources, Conservation and Recycling*, *188*, 106701. doi:10.1016/j.resconrec.2022.106701

Enzymatic fermentation

BFT fermented its own proprietary blend of enzymes for use in the hydrolysis process. The fermentation process started with preparing 10 litres of growth media and preparing the fermentor by cleaning and sterilising the vessel. A trial run was conducted to adjust parameters like temperature and pH. After these were optimised, fermentation was initiated to scale up production for a consistent enzyme yield. Throughout this process, temperatures between 40 and 60°C, oxygenation, and constant stirring were maintained, with careful monitoring to ensure quality and productivity.

Figure 9. Fermentation of enzyme cocktail created for the purpose of textile degradation.

Enzymatic hydrolysis

A total of 1.15 kilograms of ground textiles and 10 litres of fermentation broth were utilised across several vessels. Enzymes were dosed all at once to ensure uniform distribution and maximum effectiveness throughout the hydrolysis process. The total loading amount, expressed in Filter Paper Units (FPUs)[11] at 120,000 FPUs, corresponds to the concentration measured after filtration and serves as the basis for enzyme dosage. This singular dosing approach optimises efficiency and maintains consistency in BFT's enzymatic hydrolysis experiments.

To maintain precision, specific pH and temperature ranges were adhered to during hydrolysis, as guided by established literature studies. The pH was

[11] Filter Paper Units (FPUs) quantifies the concentration of enzymes. The method involves filtering the liquid containing the enzymes through a membrane filter, which traps the enzymes on its surface. The filter paper is then incubated on an appropriate nutrient medium to allow the trapped enzymes to grow into visible colonies. The number of colonies that develop on the filter paper is counted, and this count is then converted into Filter Paper Units (FPUs).

maintained within the range of 4 to 6, at a temperature range between 40 to 60°C. Additionally, 0.1 M of acetic acid was added. These parameters have been identified as optimal conditions for enzymatic hydrolysis processes, ensuring the efficient conversion of cellulose into glucose while preserving enzyme stability and activity.

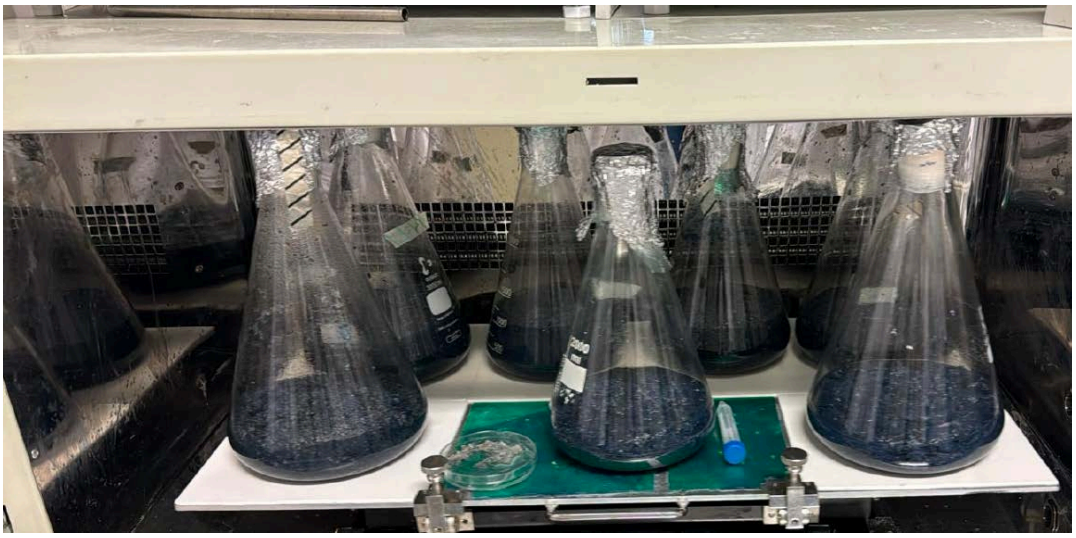


Figure 10. Enzymatic hydrolysis process (i.e. cellulose-to-glucose transformation) across multiple vessels.

Glucose and mixed remnant fibre recovery

The following downstream steps were taken to achieve a saleable glucose product, recover the enzymes, and separate and dry the mixed remnant fibre component for gasification:

- **Separation and drying of mixed remnant fibres**: The fibres were separated from the solution using a vacuum membrane filtration system. Next, the fibres were dried in an oven.
- **Filtration and concentration of filtrate**: The filtrate[12] contained enzymes, as well as dissolved or suspended solids and dyes. The filtrate was filtered twice using microfilter paper of 0.26-millimetre thickness and 1.6 µm pore size to remove microbes. It was then filtered again with a more precise filter (0.42-millimetre thickness and 0.7 µm pore size) to attempt to remove dyes. The recovered filtrate was then concentrated using a heat plate (around 60°C). Additionally, a small sample of the filtrate was separated for clarification using a charcoal filter.
- **Separation and concentration of glucose**: The resulting glucose solution produced was then filtered further through ultrafiltration using an NX

[12] The filtrate contains glucose, along with unreacted substrate and dissolved enzymes.

system, with a cutoff filter of 10 kilodalton.[13] Because the preferred in-house method of glucose concentration had a damaged vacuum pump, the glucose was concentrated using heat within a temperature range of 50 to 80°C. As a consequence of using heat-based concentration within a limited timeframe, a solid brown residue was formed on the glucose solution. This was owed to either caramelisation or the Maillard[14] reaction. This solid residue was subsequently removed, causing a loss of glucose overall. The final resulting volume of glucose was 700 millilitres.

Figure 11. The drying of mixed remnant fibres.

Proof-of-concept approach

The approach of using fixed vessels for filtering, as opposed to continuous or semi-continuous filtering, was selected by BFT for use in the proof-of-concept for several reasons:

- *Simplicity:* A single-vessel setup offers a straightforward operational model and demands less intricate infrastructure compared to continuous or semi-continuous filtering systems. This simplicity translates into potential savings in both initial capital investment and ongoing operational expenses.

[13] Ultrafiltration is a highly effective water purification method that employs a semipermeable membrane. During this process, suspended solids and larger molecules are retained on one side of the membrane, known as the retentate side, while water and smaller solutes pass through to the permeate side.
[14] An organic chemical reaction in which reducing sugars react with amino acids to form a complex mixture of compounds. This reaction is responsible for the characteristic flavour and aroma of browned food.

- *Flexibility:* Single-vessel filtration provides greater flexibility in experimental design and process optimisation. Operators can easily adjust parameters like filtration time, pressure, and filter media to enhance separation efficiency, tailoring the process to suit the unique characteristics of the feedstock.

- *Reduced equipment requirements:* With a single-vessel setup, the need for additional equipment such as pumps, valves, and extensive piping networks, which are essential for continuous or semi-continuous filtering, is eliminated. This reduction in equipment not only leads to cost savings but also diminishes maintenance demands.

- *Batch processing:* Single-vessel filtration is ideally suited for batch processing, where a finite amount of feedstock is processed at one time. This approach is particularly advantageous for research and development purposes, allowing precise control over experimental conditions.

- *Ease of operation:* Operating a single-vessel filtration system is typically more intuitive and requires less specialised training compared to continuous or semi-continuous systems. This inherent simplicity enhances process reliability and minimises downtime attributable to operator error.

- *Reduced cross-contamination:* Continuous or semi-continuous filtering systems may pose a higher risk of cross-contamination between different fractions due to the continuous flow of material. In contrast, single-vessel filtration processes each batch separately, thereby mitigating the risk of contamination between batches.

Further optimization and analysis of continuous processes will be investigated for operations at a larger scale.

Glucose analysis

The initial glucose concentration was estimated to be 300 grams per litre, and was needed as early as possible in the pilot process to form a reference point for the experimental setup of PHA production. Soon thereafter, the formal measurement was conducted by an external lab using a D-glucose assay, which found the concentration to be 63 grams per litre (44 grams in 700 millilitres).[15] From this result, a conversion efficiency of 7.82% was derived.[16]

[15] The large difference in results likely stems from the presence of nitrogen in addition to the presence of glucose-derived polymers, which arose due to the use of heat for concentration. In the absence of a definitive conclusion on which measurement is most accurate, both concentrations are taken into account in the further processing by EV Biotech.
[16] Efficiency = Actual Yield / Theoretical Yield = 44 g /562.5 g

Material losses during the experiment were calculated as follows:

$$1098.64\ g\ (final\ amount\ of\ textiles\ in\ plastic\ bag)\ -\ 10\ g\ (plastic\ bag)\ =\ 1088.64\ g$$

$$1150\ g\ (initial\ amount\ of\ textiles)\ -\ 1088.64\ (final\ amount\ of\ textiles)\ =\ 61.36\ g$$

$$61.36\ g\ of\ textiles\ transformed\ -\ 44\ g\ glucose\ obtained\ =\ 17.36\ g\ material\ lost$$

$final\ amount\ of\ textiles\ in\ plastic\ bag$ = weight of the textiles after hydrolysis (including the transportation bag)

$final\ amount\ of\ textiles$ = weight of the textiles after hydrolysis (excluding the transportation bag)

$initial\ amount\ of\ textiles$ = weight of textiles before processing by hydrolysis

If material loss from solid residue removal was excluded then the efficiency for 61 grams of textile transformed would be 10.84%.

Factors influencing the rate of hydrolysis

Several factors influenced the rate of enzymatic hydrolysis. One significant factor is the presence of inhibitory substances, such as adhesive binding agents found in composite textiles. The impact of these substances on cellulase activity depends on factors like their chemical nature, concentration, and interactions with enzymes. In the original textile sample, we observed that multilayer materials containing substantial amounts of adhesives hindered the process, requiring manual separation to reveal the adhesive's presence.

Additionally, literature studies indicate that glucose itself does not limit enzymatic hydrolysis; instead, soluble cello-oligosaccharides play a pivotal role. These compounds are rapidly hydrolysed, primarily into cellobiose, which is further converted into glucose. While the glucose saturation point has not been measured, conducting such experiments could provide valuable insights into the process dynamics.

Conclusions

The mixed textile waste stream was successfully converted into a glucose and a mixed remnant fibre stream. Based on third party testing, the conversion rate was 7.82%, yielding 44 grams from 700 millilitres of glucose with a measured concentration of 63 grams per litre. If the removal of the solid residue from glucose concentration was included and time had allowed, the calculated

efficiency could reach 10.84%. BFT believes the quantity of glucose produced was primarily limited by incomplete enzymatic hydrolysis due to the formation of soluble cello-oligosaccharides.

D4T OUTCOMES ANALYSIS - ENZYMATIC HYDROLYSIS

Enzymatic hydrolysis of cellulose containing mixed textile waste is the most critical juncture of the Rotterdam pilot in terms of innovation and difficulty. Conversion of mixed plastics to syngas and other gaseous products (methane, methanol) and glucose-to-PHA are more demonstrated pathways. Therefore, it is the first juncture of this biorecycling pathway that is most influential in understanding whether it is technically and economically feasible to harvest cellulosics from a mixed-textile waste stream versus sending all of it to become syngas. That stated, the cellulose-to-glucose transformation is a known phenomenon. Being able to do this in the presence of plastic contaminants and additional chemistries is the focus of D4T's research question, as well as understanding whether or not the output glucose is a viable biotechnology feedstock if it only undergoes minimal filtering. With these key questions in mind, D4T has the following outstanding questions and recommended next steps:

Feedstock preparation

After agreeing to engage in this pilot, the CE and D4T teams were informed that BioFashion Tech believed the textile waste streams targeted by this initiative were **all** of those that could not be reused, mechanically recycled, or chemically recycled. This would have included textile blends with high cellulose-to-synthetic fibre ratios (i.e. 50-70% cellulosic content by weight). When the textile preparation process took place, the BioFashionTech team observed a high volume of complex, synthetic-heavy blends, as well as composite materials, raising a concern about enzymatic processability. Per this concern, the BioFashion Tech team added more cellulosic material to the textile feedstock. This addition increased potential processability and yields in the trial, and still allowed us to understand enzymatic transformations in the presence of non-target textile fractions; however, it decreased our ability to gain insights into whether or not the **lowest-value** waste streams could be processed both technically and economically. For the next phase of this pilot, D4T intends to utilise feedstocks that are representative of more specific waste fractions—both mid-tier unrecoverable waste (such as those listed above, with 50-70% cellulosic content, some of which have existing markets) and the lowest value fraction (containing more composite materials, most of which go directly to local incineration).

Yield - limiting factors

Many of the limitations of the pilot are related to the design of the experiment at lab scale to successfully produce waste-derived glucose. BFT calculates that it obtained a yield of 7-10%. This seems to be a very low yield, even at lab scale. We attribute these low yields to:

1) No pretreatment of the cellulosic fraction to open up the crystalline structure. The literature points to this as a critical step, as well as the need to find less chemical and energy intensive forms of pretreatment.
2) Single batch processing versus a continuous flow *and* a filtration process to separate glucose product and/or non-cellulosic materials from the target fraction as soon as possible. Single batch processing might allow for less enzyme loading initially, but their effectiveness *could* be diluted due to the interference of non-target material that inhibits enzymatic accessibility to target fractions. We would be interested to see what impact a continuous processing would have on yields, and recommend scaled processing in alignment with industry best practices.
3) Impacts of colourants, antimicrobials, surface treatments, and adhesives are reported to affect the effectiveness of hydrolysis. The experiment did not examine what chemical substances were present pre-hydrolysis, nor what substances may have been released post-hydrolysis, from both target and not-target fractions prior to further processing. We would devise a method for this analysis in future research.
4) Not assessing what substances may have transferred from the textile waste to the glucose prior to filtration, thus it is unclear how much filtration of textile waste-derived glucose may require because we did not methodically test for this variable. The cost of filtration and purification at a commercial scale is a fundamental, but open, question. We would include that testing, and the exploration of various filtration methods, in future research.

Techno-economic viability

Although we have proven *some* aspects of the technical viability of mixed-waste conversions, as this pilot scales, we will explore this in relation to the economic viability of enzymatic hydrolysis to this end. We would like to evaluate if there is a theoretical minimum percentage of cellulosic materials required to make enzymatic hydrolysis economically viable for glucose production, and whether or not the market price of glucose is sufficient to rationalise the use of this production method. Similarly, we'd like to investigate the possibility of increasing profitability if the same facility (e.g. a biorefinery) could convert the glucose to higher value platform chemicals. A key question is whether or not the 7-10% yield obtained in the experiment warrants further research before running trials in a commercial scale bioreactor in order to gather data to judge the economic and technical viability of this method of biorecycling.

2.4 BACTERIAL FERMENTATION

Research questions

The glucose solution produced by BFT was sent to EV Biotech to be used as a feedstock for PHA production. For this processing step, we set out to answer the following research questions:

- Can microbes grow on glucose derived from mixed post-consumer textile waste?
- Can microbes produce PHA from this glucose?
- How comparable is textile waste derived glucose to commercial grade glucose?

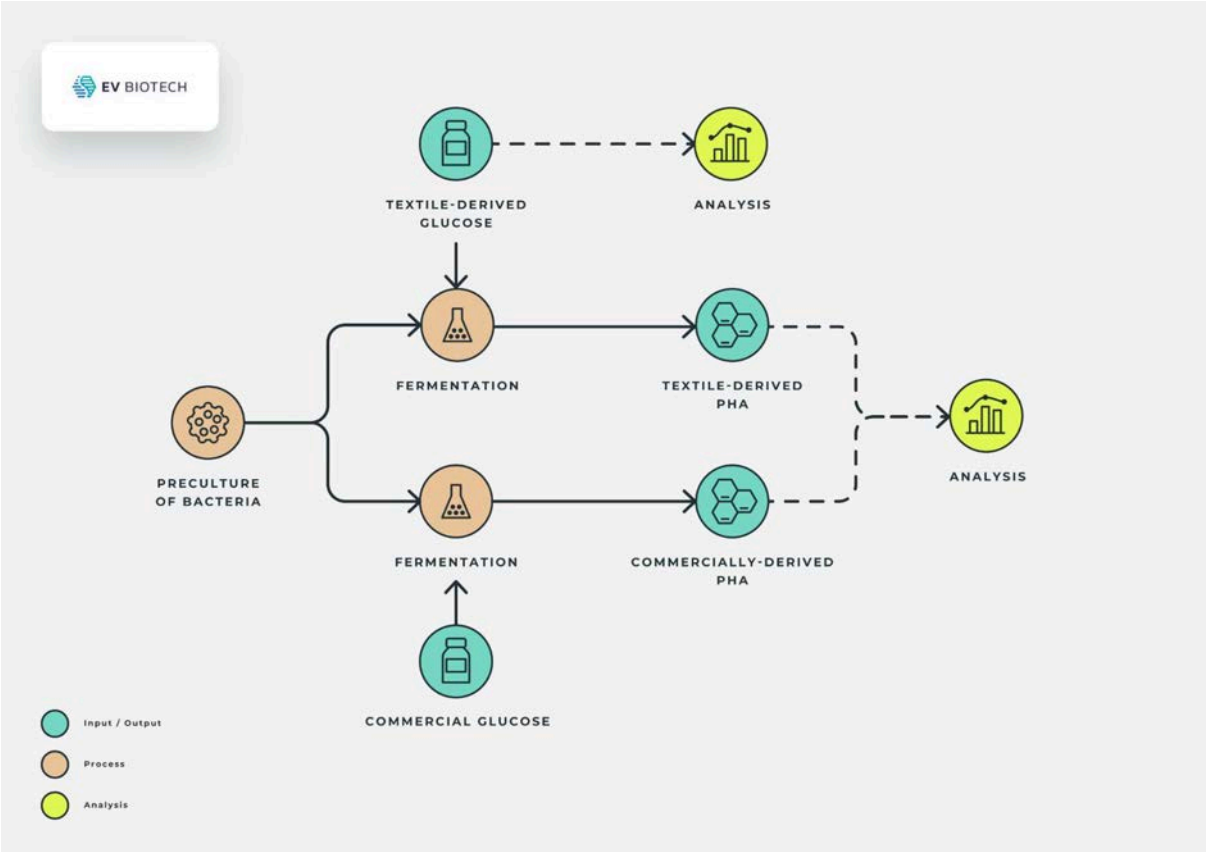


Figure 12. Schematic diagram of the process steps taken by EV Biotech.

Bacterial fermentation

Experimental setup overview

The experiment focused on cultivating and utilising two types of gram-negative, mesophilic bacteria,[17] namely *Escherichia coli (E. coli)* and *Pseudomonas putida (P. putida)*, for the synthesis of polyhydroxyalkanoates (PHAs). PHAs are biopolymers with potential applications in various fields, including bioplastics and textiles production.

Organisms and PHA production:

PHAs were produced using both synthetic (plasmid-based) and natural (wildtype strain) organisms. The induction of PHA synthesis occurred via chemical induction with IPTG (Isopropyl β-D-1-thiogalactopyranoside) for *E.coli* and through nutrient limitation, specifically nitrogen depletion for *P. putida*.

Control and sampling:

A negative control for *E.coli* involved a strain with an empty plasmid, ensuring that PHA production was not influenced solely by the presence of the plasmid.[18] A negative control for *P.putida* involved taking samples during the late exponential phase of bacterial growth.

Media and conditions:

The experiment was conducted in shake flasks using defined mineral media. Two distinct media formulations were employed: EV-M-D1 and EV-M-D21. These media offered different nutrient compositions or concentrations, providing insight into how varying environmental conditions affect PHA production.

Organism	***Escherichia coli***	***Pseudomonas putida***
PHA produce	Synthetic (plasmid-based)	Natural (wildtype strain)
Induction of PHAs	Chemical induction (IPTG)	Nutrient limitation (nitrogen)
Negative control	Strain with empty plasmid	Samples in late exponential phase
Other requirements	Kanamycin for plasmid maintenance	-

Table 13. Overview of experimental bacteria utilised and their associated requirements.

[17] Mesophilic bacteria are a type of bacteria that thrive in moderate temperature conditions, typically between 20°C to 45°C.
[18] To maintain the plasmid carrying genes for PHA synthesis, kanamycin is included in the growth media.

Outcomes analysis

Various analytical techniques were employed to assess key parameters: cell growth, glucose consumption, and PHA accumulation. These techniques helped to understand the dynamics of bacterial growth and the production of PHAs under different experimental conditions.

	Cell growth	**Glucose consumption**	**PHA accumulation**
	Assessment of cell density	*Assessment of free D-glucose*	*Assessment of PHA by Nile Red*
Method	Spectrophotometer	GL6 metabolite analyser	Microplate reader
Output	Optical density (OD600)	glucose in % w/v	Semiquantitative by relative fluorescence units (RFU)
	Assessment of dry biomass		
Method	Analytical balance		
Output	Dry cell weight (DCW) in g/L		

Table 14. Overview of methods and outputs for each part of the analysis.

Before conducting the experiment, the textile-derived glucose was filter-sterilised to eliminate potential microbial contaminations, and concentration measurements were performed. These steps were conducted with different variations of the textile-derived glucose using several different methods, summarised in *Table 15*. Following this, samples were prepared by dilution to yield samples of 20 grams per litre. Because the concentration of the textile-derived glucose stock ranged between 40 and 300 g/L, three different stock concentrations were assumed for the preparation of the medium. These concentrations were set at 300 g/L, 150 g/L, and 50 g/L to encompass a variety of glucose levels. It was known that the in-house measurements detected only free D-glucose, but not any glucose-derived compounds (i.e. oligomers). It was thus assumed that the total carbon (free glucose + glucose-derived compounds) was higher than the in-house measured results. The final medium was targeted to contain a glucose concentration of 20 g/L (*E. coli*) or 15 g/L (*P. putida*), for which the required volume of the stock solution was calculated. Subsequent measurements of the concentrations in the final medium indicated variations depending on the quantity of glucose stock added to the medium. A comparison

of how the concentrations looked visually compared to commercial glucose and their measured concentrations is provided in *Figure 16*.

**Two tests were conducted and an average was taken. In all cases, the results were very similar.*

Treatment/test	Method	Concentration
Unpurified	GL6	39.7 g/L*
	Strips[19]	43.7 g/L*
Charcoal-purified	GL6	57.9 g/L*
	Strips	62.3 g/L*
BFT Initial Estimate	D-glucose assay	300 g/L
External analysis[20]		63 g/L

Table 15. Pretreatment methods and measurements of textile-derived glucose.

[19] A strip-based glucose monitoring system measures the glucose level in a liquid sample. This was conducted as a second, independent test of the initial glucose measurement results.
[20] Issued by BioFashionTech

Commercial

Glucose = 18.0 g/L

Low concentration

Glucose = 4.7 g/L

Medium concentration

Glucose = 9.3 g/L

High concentration

Glucose = 30.3 g/L

Figure 16. Comparison of commercial glucose to textile-derived glucose at different levels of concentration.

Analysis results

The analysis was conducted using 50-millilitre samples of glucose with a concentration of 20 grams per litre at a temperature of 30°C and shaking at 200 revolutions per minute.

Observations on *E. coli*:

- Growth in the 'low' and 'medium' glucose conditions showed comparable results to commercial glucose. The ‘medium’ solution displayed results exceeding that of commercial glucose. There was no observable growth in the 'high' glucose condition.
- Complete consumption of glucose was noted in the 'low', 'medium', and commercial glucose conditions.
- PHA accumulation was observed in the 'low' and 'medium' glucose conditions after 22 hours of incubation, exceeding that of commercial glucose.

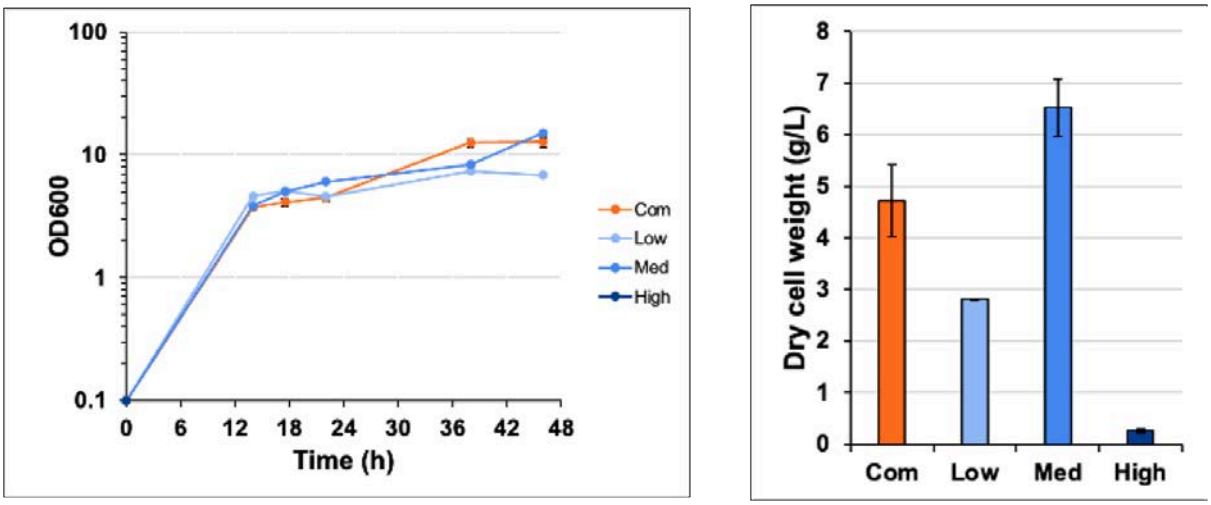
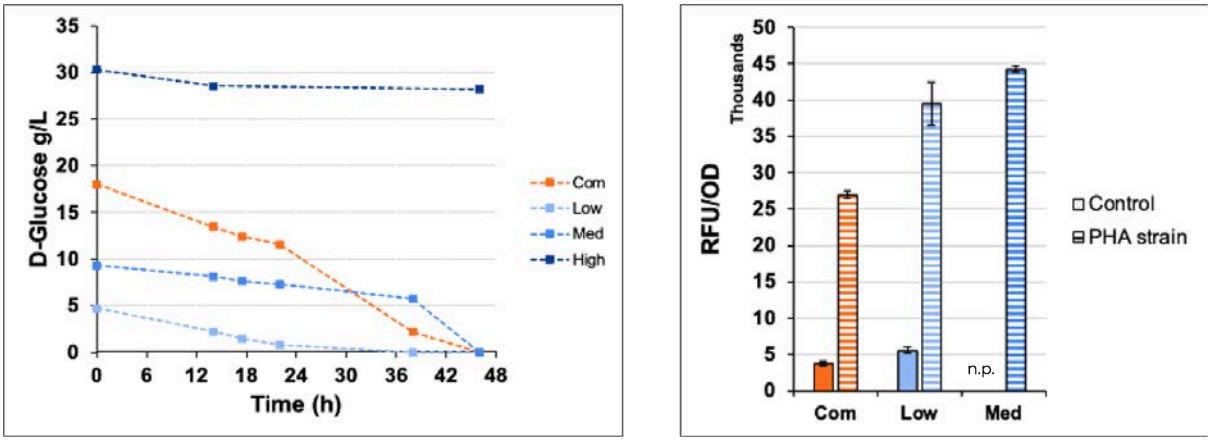


Figure 17. *E.coli* growth curve.



n.p.: not performed

Figure 18. *E.coli* glucose consumption and PHA accumulation.

Observations on *P. putida*:

- Growth in the 'low' and 'medium' glucose conditions exhibited comparability with commercial glucose, with the ‘medium’ solution yielding exceptionally good results. While no discernible growth was observed in the 'high' glucose condition.
- Textile-derived glucose was rapidly and completely consumed by the bacteria.
- PHA accumulation was achieved in the 'low' and 'medium' glucose conditions, showing similar trends to those seen with commercial glucose.

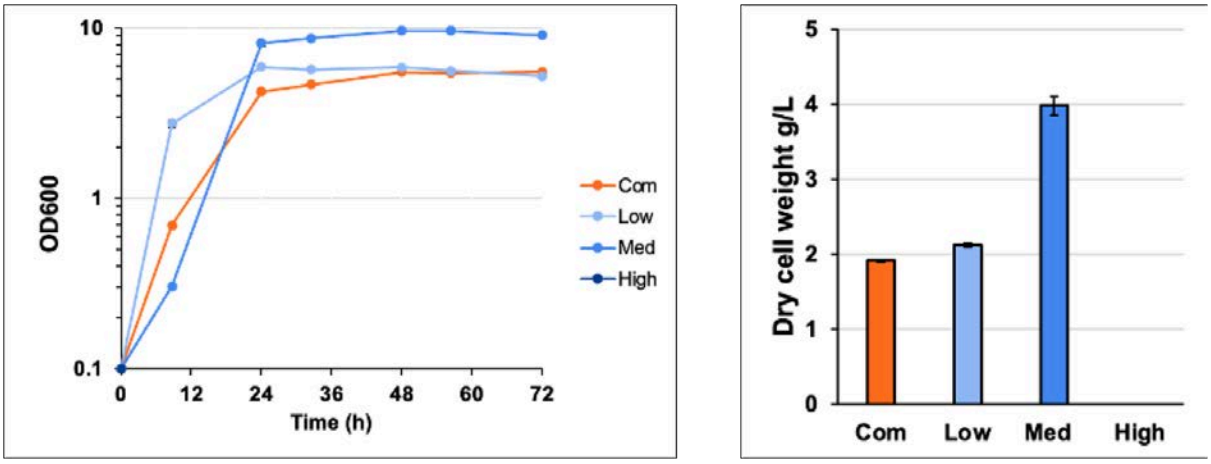


Figure 19. *P.putida* growth curve.

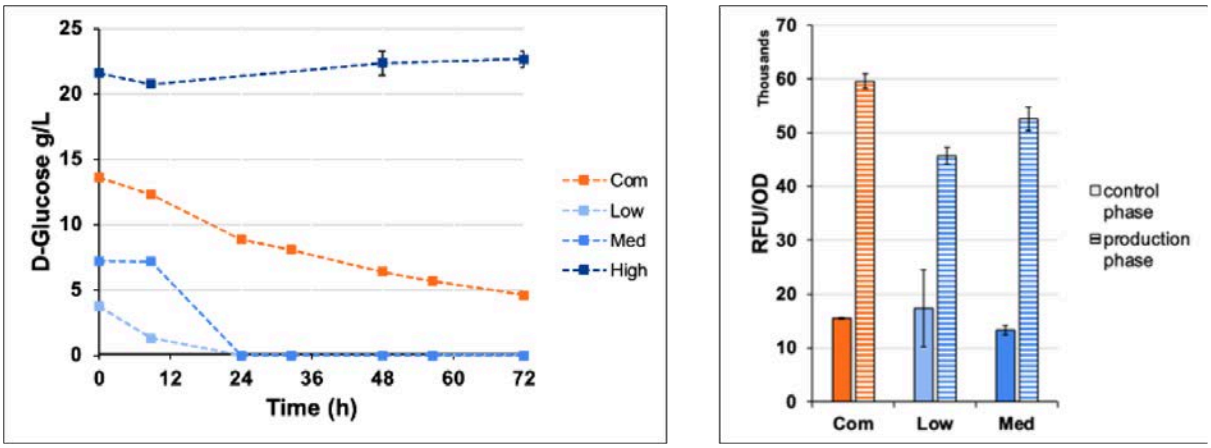


Figure 20. *P.putida* glucose consumption and PHA accumulation.

Conclusions

Based on the findings from the experiments, it can be concluded that both textile-derived and commercially sourced glucose exhibited similar outcomes in terms of bacterial growth and PHA production. These pilot results suggest that textile-derived glucose performed equally or even exceeding that of commercially available glucose for supporting microbial growth and facilitating PHA synthesis. The 'medium' concentration proved most effective and provides a promising starting point for further optimisation. Moreover, it must be noted that the presence of dyes[21] in the glucose solution did not prevent successful PHA synthesis.

[21] In this phase of the pilot, the presence of residual dyes and chemistries was not analytically tested. This conclusion assumes that some residual dyes and chemistries were present in the glucose due to the low levels of filtration in previous steps.

D4T OUTCOMES ANALYSIS - BACTERIAL FERMENTATION

Market Value

One of the most significant potential barriers to the adoption of textile-derived glucose is its cost in comparison to the easily accessible and well developed industry for converting starches to commodity glucose—the impacts of which are (per our understanding) a hotspot in life cycle analyses (LCAs) of the budding biomaterials market. Further research will explore a reasonable target price for textile-derived glucose as compared to commercial equivalents or as inputs for innovative biomimetic materials like those produced by EVB and textile industry leaders, such as Spiber.

Genetic Modification - Adoption

Although there was notable success with both the wild and synthetic bacterial strains, we'll need to explore the associated commercialization impacts of utilising "naturally occurring" vs "genetically modified" strains in relation to emerging European and International policy, brand or manufacturer requirements, and consumer sentiment.

Genetic Modification - Performance

Similarly, we'll need to compare the differences in yields across wild and synthetic strains with an ability to synthesise the physicochemical performance properties required to meet material- and product-level quality, manufacturing, and durability standards for specific applications. Given D4T's understanding of the "state of the state" with regard to PHA R&D in apparel, EVB is looking to partner with a regional producer of agri-textiles, which are ripe for innovation in mitigating future fibre fragmentation and microfiber/plastics pollution.

Quality Control & Contaminants

Aside from consumption, growth, and performance metrics, there are a variety of quality-related factors that may benefit or inhibit textile-derived glucose use in an industrial setting. To meet both technical and economic requirements, industry must strike a balance between the cost of filtration and the downstream effects of glucose that isn't purified to current commercial standards. Moving forward, we will partner to better understand the differences between commercial and textile-derived glucose, and what steps are necessary to address these differences. This will require the exploration of limiting factors for feasible production in large-scale scenarios (to avoid cross-contamination), and gaining a better understanding of the types of substances biotech partners would be monitoring which would affect production conditions and viability (such as dyes, antimicrobials, or PFAs). We were unable to test the variables directly during the proof-of-concept phase.

2.5 GASIFICATION

The mixed remnant fibres produced by BFT are sent to TNO to be gasified into product gas. The targeted product gas was all non-condensable gases (as opposed to the condensable gases). For this processing step, we set out to answer the following research questions:

- What are the resulting products generated by gasifying a predominantly mixed remnant textile waste stream, with less (or the complete absence of) cellulosic content?
- How do variations in feedstock and operating conditions impact the product gas composition?

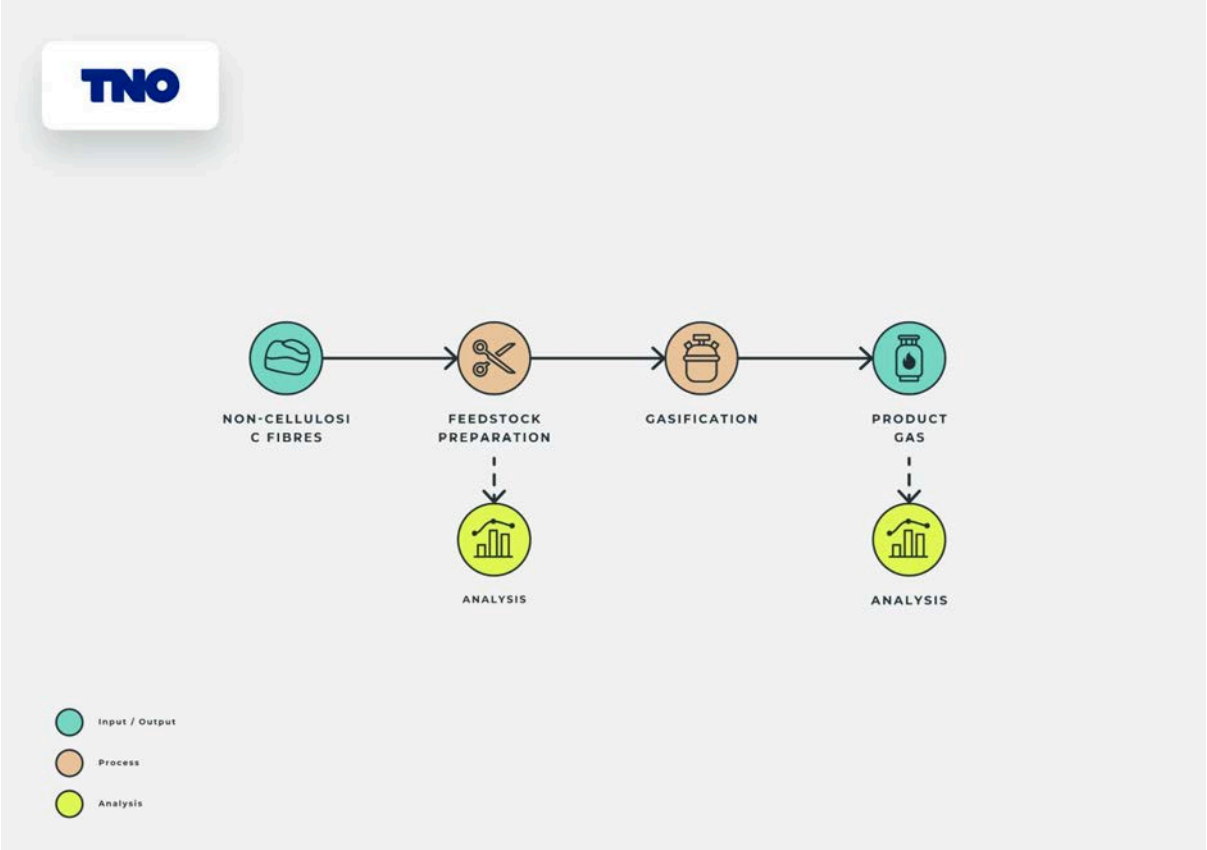


Figure 21. Schematic diagram of the process steps taken by TNO.

Feedstock preparation

The residual—primarily plastic—stream from BFT was received as a dry solid, weighing approximately 1 kilogram. This material was put through a pelletiser, however, given the nature of the material,[22] pellets were not able to be formed. Instead, the stream was characterised as shredded plastic.

[22] Textiles don't produce traditional pellet shapes due to their fluffiness.

Given the scale of the gasification reactor, it was deemed necessary to have more material to perform separate tests, and thus a separate fraction of “unprocessed” textile waste from Erdotex was used. This fraction of textile waste was manually sorted to consist of primarily synthetic (i.e. fossil derived) fibres, which was theoretically equivalent to the expected composition of outputs from BFT. The fraction was subsequently put through a pelletiser to produce shredded synthetic fibres, comparable in form to the BFT residual textile stream. These pre-processing steps were necessary to ensure compatibility with the feeding system of the gasifier.

Figure 22. The shredding machine (left) and resulting shredded output (right).

Figure 23. depicts the pelletising machine (left) and resulting shredded output (right).

Feedstock analysis

Elemental analysis was used to determine the composition of: (1) the sorted **synthetic** textile waste stream sourced from Erdotex, (2) the textile waste processed by **BFT**, (3) a mixed textile waste stream sourced independently by **TNO**, (4) a pure PET fibre stream, and (5) a pure nylon fibre stream. *Table 24* describes these streams further.

Fibre Stream	Testing label	Description
Synthetic feedstock	STW	Textiles sourced from Erdotex that were sorted to only contain synthetic fibres.
BFT feedstock	BFT	Mixed remnant fibre output from BFT (reduced cellulose)
TNO textile	TNO	Textiles sourced by TNO, containing an nonspecific[23] mixed composition
PET	-	Textiles sourced by TNO containing only PET-based fibres
Nylon	-	Textiles sourced by TNO containing only nylon fibres

Table 24. Description of analysed feedstocks

Table 25 displays the results of the elemental analysis for above-listed fibre streams. *Figure 26* displays the elemental analysis specifically of the inorganic elements for the three different mixed textile waste streams.

Analysis	Units	STW	BFT	TNO	PET	Nylon
Br	mg/kg db	187	29	34	< 10	< 10
Cl	mg/kg db	1727	474	546	28	23
F	mg/kg db	20	< 10	< 10	< 10	< 10
Ash (550)	mg/kg db	0.8	1.7	1.4	0	0
Volatiles	% db	85	84	88	94	100
Humidity	% ar	< 1	n.d.	5	0	0
High Heating Value	MJ/kg db	24	20	21	23	31
C	% db	61.0	52.7	54.8	62.4	62.4
N	% db	2.3	0.8	1.8	0.0	12.0
H	% db	4.8	5.4	5.6	4.3	9.8
O	% db	30.3	39.7	32.2	33.2	16.2

Table 25. Elemental analysis of five different textile feedstocks.

[23] For this textile fraction, TNO did not collect composition data from clothing labels before undergoing the process of shredding and elemental analysis

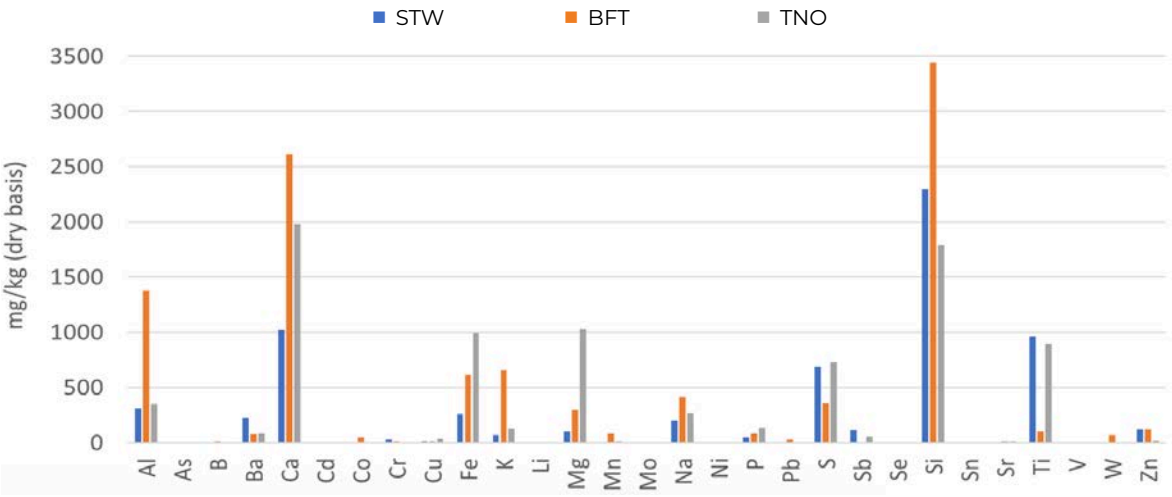


Figure 26. Elemental analysis of inorganic elements of three different textile waste streams.

In the analysis, synthetic textile waste (STW) was estimated to be made up of approximately 3% cellulose content by weight, while BFT feedstock comprised around 34 wt%. It's important to note that this method has not been validated for textile samples and should only be considered as an *indication* of cellulose content.[24]

Gasification

A gasifier is a reactor system designed to convert solid waste materials, such as biomass and plastics into primarily gaseous products through a high-temperature thermochemical process. This process involves heating the feedstock in a controlled environment with a restricted supply of oxygen or air, leading to partial oxidation and decomposition. TNO utilised their WOB fluidized bed reactor for the pilot. This is a flexible reactor capable of processing diverse feedstocks at rates of up to 500 grams per hour and temperatures ranging from 150 to 1000°C. This gasifier accommodates various carrier gases (such as air, steam, oxygen and nitrogen) and is compatible with a range of feedstocks including lignocellulosic biomass, grass, manure and plastic waste.

[24] This estimation, conducted by TNO, measures glucan which is assumed to be generated by the presence of cellulose in the feedstock.

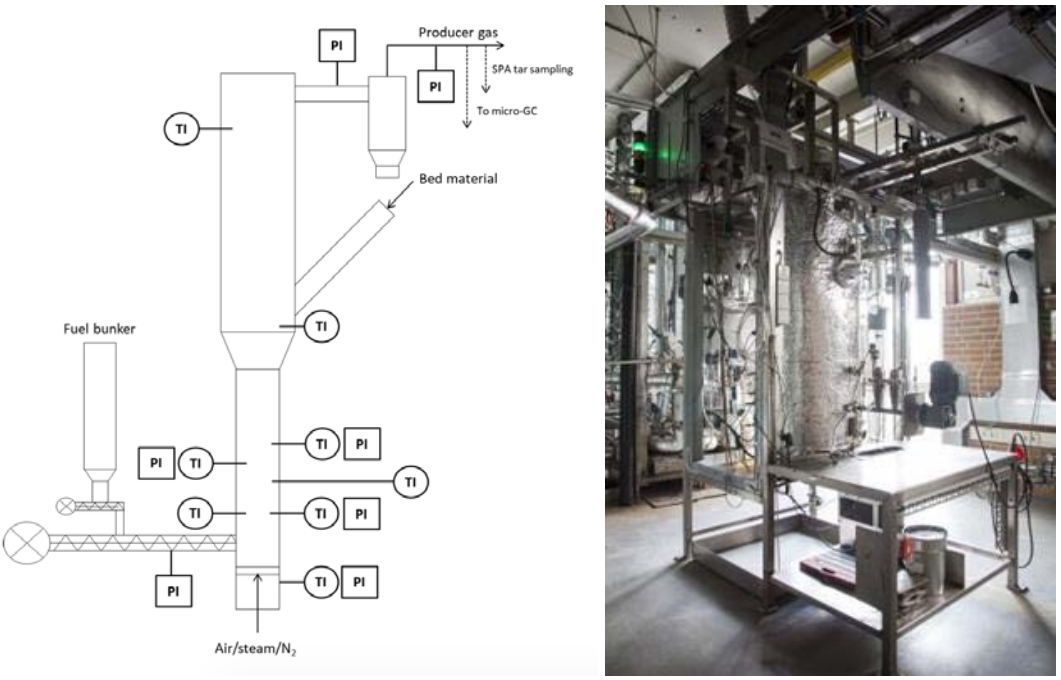


Figure 27. Schematic diagram of the gasification reactor (left) and image of gasification reactor (right).

Several different tests were planned to be completed, with both the separated synthetic textile waste (STW) and the processed textiles from BFT. *Table 28* displays the different operating conditions selected for each test run, with variations in temperature,[25] bed material,[26] feed flow, steam, nitrogen (N2) and Neon (Ne)[27].

		STW	STW	STW	STW	BFT
Temperature	°C	810	880	810	880	880
Bed material	-	sand	sand	olivine	olivine	olivine
Feed flow	g/h	90	90	90	90	90
Steam	g/h	100	100	100	100	100
N_2	NL/min	9	9	10	10	9
Ne	NmL/min	20	20	20	20	20

Table 28. Operating conditions of five different test runs for gasification

[25] Temperatures below this were not tested due to the knowledge that lower temperatures would not yield the desired product gases, as opposed to BTX, for example.
[26] Bed material refers to the solid particles that form the bed within the gasifier reactor. This bed serves as a support medium for the feedstock material and provides a surface for the gasification reactions to occur. The choice of bed material can influence the gasification process by affecting heat transfer, reaction kinetics, and the composition of the resulting gases.
[27] Neon is used as a TNO standard to allow for mass balance calculations.

The BFT stream, amounting to around 1 kilogram, posed feeding challenges due to insufficient volume, resulting in operational issues. Insufficient material in the feeding screw hindered traction, impeding the material's transfer into the reactor. Ultimately the issue was resolved by removing the larger pieces with a sieve, which resulted in discarding around 10wt% of the material.[28] *Figure 29* displays the blocked entrance of the feeding entrance and the different feeding screws used to try to resolve this issue.

Figure 29. The blocked entrance in the feedstock bunker (left), three different feeding screws (right).

A mixture of steam and nitrogen served as the carrier gas in the experiment. The inclusion of nitrogen as a carrier gas was essential due to the low material feed rates. Consequently, the resulting product gas became diluted with nitrogen.

Gasification analysis

Analysis of the gasification output contained both non-condensable and condensable gases,[29] each fraction with their own analytical methods. *Table 30* displays the analytical method applied to each fraction.

[28] This issue did not arise in previous tests conducted with textiles, where larger volumes were used.
[29] Condensable gases readily condense into liquid or solid states with small changes in temperature or pressure, while non-condensable gases remain in the gaseous phase under typical conditions without undergoing condensation.

Product gas fraction	Analytical method
Non-condensable gas	• Online gas analyser (H2, CO, CO2, CH4, O2) • Online compact-GC (Ne, H2, CO, CO2, CH4, C2H2, C2H6, C3H6, C3H8) • Off-line GC-FID (C1-C6 hydrocarbons and H2)
Condensable gas	• Guideline (aromatics including benzene and toluene)

Table 30. Analytical methods performed on each product gas fraction.

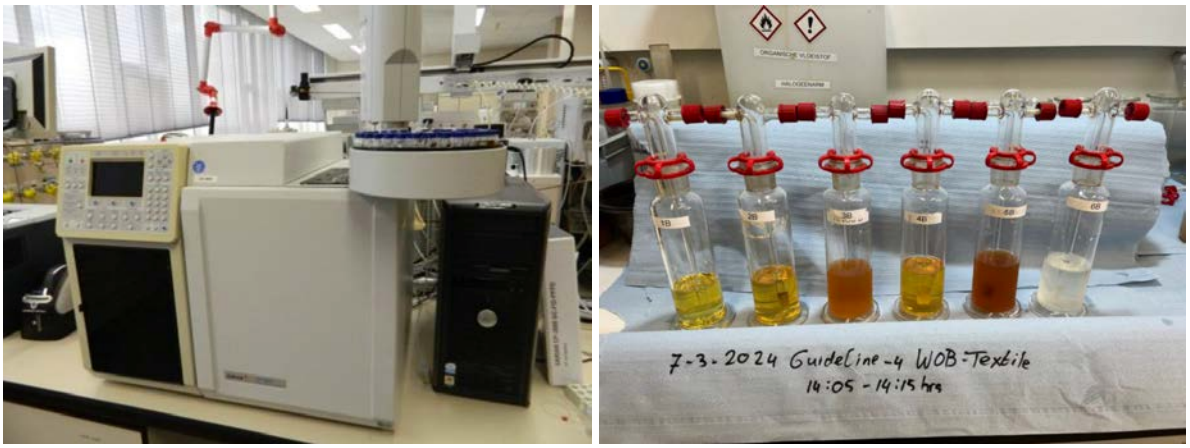


Figure 31. Off-line GC-FID machine (left) and guideline method for quantifying condensable gas (right).

The **non-condensable gas** fraction was found to be dominated by carbon monoxide (CO), carbon dioxide (CO2), hydrogen (H2) and methane (CH4), with smaller fractions of ethylene (C2H4) and acetylene (C2H2). The results for all five test runs are displayed in *Figure 32*.

Analysis of the **condensable** fraction showed that the main component produced in all test runs was benzene, followed by significantly smaller fractions of toluene, o-Xylene, styrene, naphthalene and biphenyl (displayed in *Figure 33*).
Overall, the **main product gas categories** produced were non-condensable gases (45 to 61%), followed by benzene and toluene (11 to 25%), and tars[30] (2 to 9%).[31] The main product gases (irrespective of their condensable or non-condensable nature) were CO, CO2 and H2 across all five test runs. These are summarised in *Figures 34 and 35*, respectively. After each test air was injected into the system to burn any solid state carbon (char) that was in the bed or within the pipelines. This was accounted for by quantifying the CO2 released.

[30] Tars are defined here as aromatic compounds with a higher molecular weight than benzene.
[31] These proof-of-concept calculations are subject to uncertainty due to the low feedstock quantities and high levels of dilution.

It was found that higher temperatures lead to more syngas (CO, CO2, H2 and CH4) produced, less higher hydrocarbons (C2+), and less tars. They also reduce benzene, though to a lesser extent. The tar catalytic effect of olivine was rather limited at the tested temperatures.[32] However, it did lead to an increase in CO2 and H2 in the product gas. The BFT material, which contained more cellulosic materials, showed a clear reduction of tars and benzene in the product gas obtained as well as a higher share of H2.

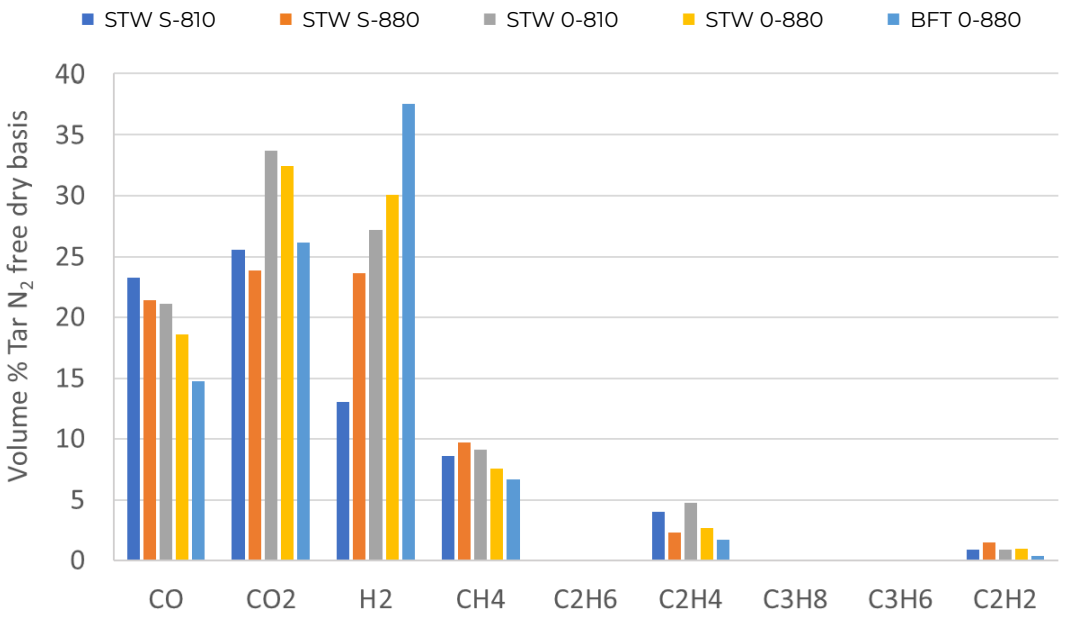


Figure 32. *Non-condensable gas* fraction produced from gasification.

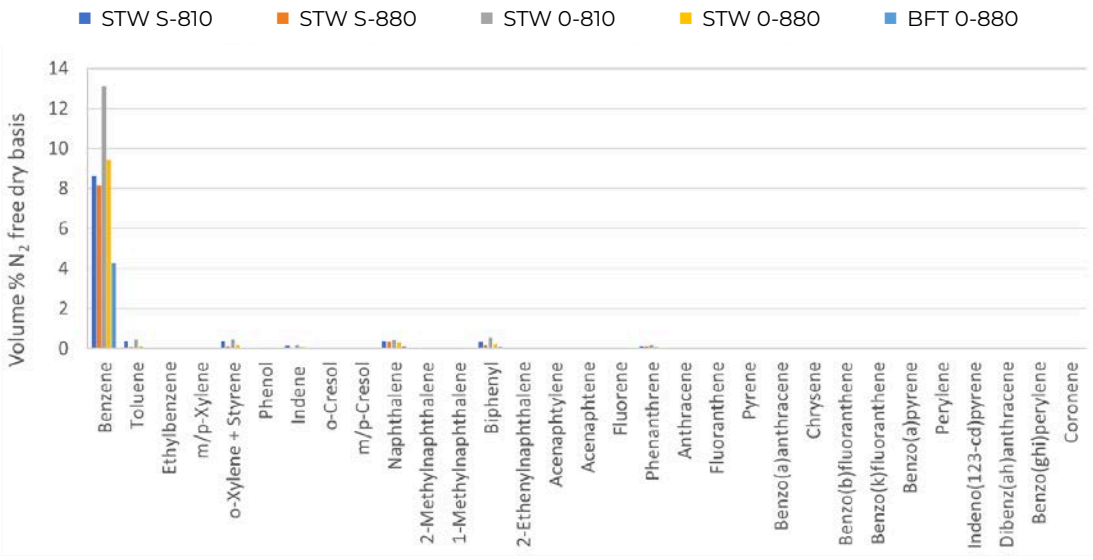


Figure 33. *Condensable gas* fraction produced from gasification.

[32] Olivine is commonly used as bed material due to its tar cracking capabilities.

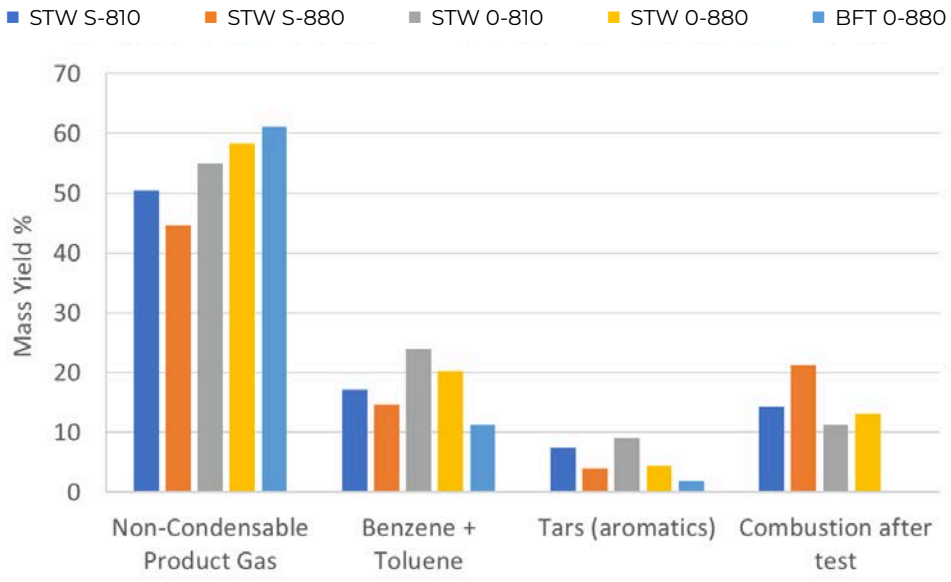


Figure 34. *Main product categories* produced from gasification.

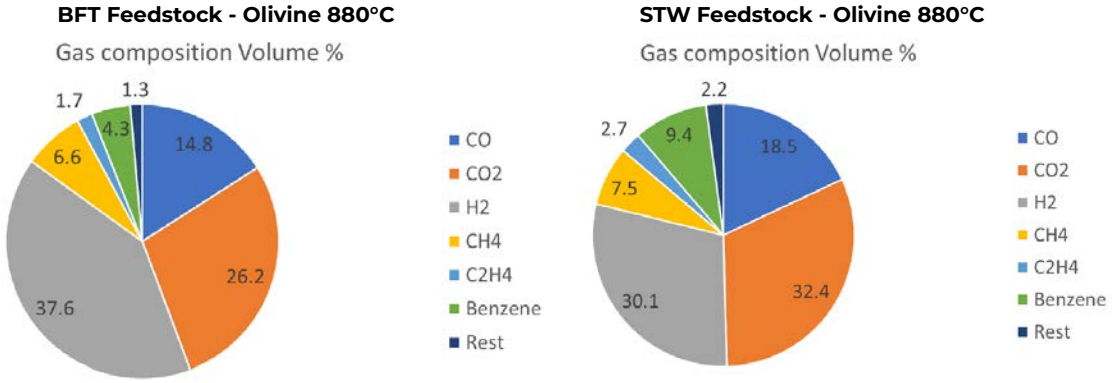


Figure 35. Main gases produced by two of the test runs.

The gasification also produced a fraction of solid-state carbon. This solid fraction was combusted after the testing in order to quantify its presence.

Conclusions

At the conditions examined, the predominant output is the product gas, with conversion rates exceeding 70 wt%. This product gas primarily consists of H2, CO, and CO2, constituting approximately 80 vol% in tests utilising olivine. Furthermore, at the temperatures tested, all high hydrocarbons (>C2H4) are effectively converted, while benzene is present at significant levels, accounting for 20 wt% in plastic textile gasification (olivine at 880°C) and 10 wt% in BFT gasification (olivine at 880°C). The composition of the product gas varies depending on the feedstock and gasification conditions employed.

D4T OUTCOMES ANALYSIS - GASIFICATION

Preferred Outputs

As stated previously, the intention of this pilot was to transform unusable waste into regionally-relevant nutrients (i.e. feedstocks) in support of the emerging bioeconomy. We have proven the opportunity for converting mixed textile waste to biotech inputs is technically possible; however, syngas production is also used to produce commodity chemicals—such as benzene, toluene, and xylene (BTX), which have defined market value but which are also more toxic than their biomimetic alternatives. Given the influence and existing footprint of the petro-chemical industry, the economic incentives are aligned with producing outputs that can be easily folded into petro-chemical supply chains. Our goal is to create outputs that build biomimetic supply chains. In the next phase, we will work closely with local stakeholders and techno-economic experts to build a broader business case for the pursuit of safer chemistry, materials, and systems that can contribute to building the bioeconomy

Solid State Carbon

TNO's gasification process also yields solid state carbon materials. In the next phase of work, we'd like to further explore the highest and best use for this form of carbon. For example, uses range from generating energy for the gasification process, a soil supplement for use in regenerative agriculture, the electrification of our energy systems, dyes and pigments, and/or other values-aligned applications.

Exploring the Impact of Cellulose

There were many research questions related to this portion of the transformation pathway, including whether or not the presence of cellulose greatly affected the desired outputs. The intention was to compare a non-cellulosic fraction (i.e. the resulting fibre fraction left after enzymatic hydrolysis) of waste against TNO's previous mixed textile waste studies. Although low enzymatic hydrolysis transformation rates and lab-scale processing introduced difficulties in making 1:1 comparisons across feedstocks, interesting insights were garnered that should be further explored when considering textile-waste-to-biotech pathways, such as the following:

- Samples with cellulosic materials showed a "clear reduction in tars and benzenes". Results showed that the cellulosic fraction produced less than half the amount of benzene than did the all synthetic fraction.
- Cellulosic samples increased production of H2

As one of D4T's hypotheses is that low value, mixed textile waste might be a good feedstock for biorefineries and could be combined with various forms of

biomass. These insights are worth noting when considering a comprehensive future-state pilot design. Similarly—although not tested in this phase of the pilot—syngas and other gaseous products also offer an alternative source of carbon for various microbial communities compatible with fermentation processes. For example, PHA can be produced using methane instead of sugars (e.g. Mango Materials). This can also be seen in our German pilot with the theoretical use of CO2 in microalgae cultivation.

3. SUMMARY OF FINDINGS & INSIGHTS

The goal of the proof-of-concept pilot was to process mixed composition textile waste that cannot be reused or recycled through a system that creates environmentally benign, economically valuable outputs. The pilot successfully transformed a small quantity of this waste stream into several usable desired outputs, namely: glucose (as an intermediate), PHA, and a gaseous mixture mostly containing syngas.

The results prove that the known phenomena of cellulose-to-glucose conversion typically studded in pure waste streams[33] can also occur in more complex feedstock compositions, albeit at a low conversion efficiency (7.8%). There are several hypotheses as to why this may have been the case. Firstly, conducting the experiment as a batch process meant that total glucose transformation may have been inhibited by the presence of existing glucose, as it may have more readily promoted glucose to be further transformed into glucose-oligomers. Secondly, the measured concentration of glucose referred to only D-glucose, not the likely presence of glucose-oligomers, which were imperceivable to the chosen assessment method and thus not included in the conversion efficiency calculation.

The textile-derived glucose was found to be suitable for the subsequent production of PHA using two different microbial strains. However, because glucose concentration could not be accurately measured due to solid residual formation during the concentration process—indicating the occurrence of chemical reactions and thus a change in bioavailability—three different control medium concentrations were used (low, medium, high). For a precise side-by-side comparison to commercial glucose, the exact glucose concentration would be required.

The production of PHA varied depending on the assumed glucose concentration, and without further experiments we cannot say for certain why the different

[33] Rehman, N., Arain, M.B., & Shah, N. (2015). *Conversion of Cotton to Glucose by Base Hydrolysis Using Various Hydrolytic Conditions*.

concentrations led to their respective results. There could be several factors affecting the performance of the “high concentration” sample. The main hypotheses are: (1) the total carbon in the system was too high. If the system is overloaded with carbon, it might inhibit the organism e.g. through osmotic pressure. (2) there could have been growth-inhibiting substances in the textile-derived glucose. In lower concentrations, they might not have an effect, but when more concentrated, inhibit the growth. Overall the results could be due to a combination of both factors, a mix of growth-promoting and growth-inhibiting components, present in the textile-derived glucose. In medium-to-low concentrations, the growth-promoting components may have accelerated the growth. However, in high concentrations, the effect of the growth-inhibiting components may have been too strong. In any case, regulations tied to the use of textile-derived PHA for final products, applications, and target markets may require higher levels of guaranteed purity. This factor should be explored in further work.

Finally, because the gasification of mixed textiles into condensable and non-condensable gases has already been proven at TNO, it was particularly interesting to focus on textiles with low levels of cellulose and optimise this towards condensable gases (namely, CO, CO2, H2 and CH4), which are generally less toxic than condensable gases such as benzene. It was found that higher temperatures lead to more condensable gases at conversion rates exceeding 70% by weight, resulting in less higher hydrocarbons (C2+) and non-desirable tars.

Overall the pilot proved what it set out to, but there is room for improving efficiencies, measurements, and optimising outputs to match desired end products.

4. PARTNER RECOMMENDATIONS

This proof-of-concept pilot marks the initial phase in scaling and refining a system designed to effectively manage unsorted, complex fractions of textile waste. Each technology partner has contributed recommendations tailored to their expertise, laying the groundwork for advancing towards large-scale solutions for textile waste management in support of the bioeconomy.

BioFashionTech provided short-term and long-term strategies for optimising the hydrolysis of mixed fabric textile waste. Short-term objectives should be to improve enzymatic hydrolysis by exploring different pretreatment steps to increase the accessibility of enzymes and therefore boost efficiency. The use of a slurry reactor for the enzymatic hydrolysis process could also be a logical next step. This reactor's proficiency in handling solid-liquid reactions could significantly enhance reaction rates and product yields. Through improved mixing and heat transfer, the slurry reactor promises heightened operational efficiency and a more controlled reaction environment. The filtration of glucose could also be improved through the use of membranes such as granular activated carbon and zeolite filters, which are already available for wastewater treatment. Wageningen University (WUR), who currently functions as one of the pilot's biotechnology advisors, has experience in the field of filtration and so would be a useful partner here. Long-term objectives include further refining the hydrolysis process to handle different types of textile waste and enhancing the scalability of the technology.

Key learnings from the proof-of-concept pilot include that BioFashionTech technology was able to valorize a type of input that didn't have any other purpose. The efficiency gained in this process was a direct consequence of feedstock received. Thus different types of feedstocks as well as their origin, industrial or post-industrial, will lead to different outcomes—as already proved in other experiments and the scientific literature. In terms of finding the best final use of glucose, there may be a higher economic value (hundreds to thousands of US$ per tonne) from producing glucose-derived chemicals, such as levulinic acid, succinic acid, and itaconic acid. Therefore, a partnership to co-develop value-added chemicals would be valuable, as a separate initiative to PHA production.

EV Biotech provided short-term and long-term strategies for optimising the production of polyhydroxyalkanoates (PHA). Short-term objectives include refining the induction of PHA production and limiting PHA degradation through various means, such as nutrient limitation or medium optimisation. In the long term, the focus shifts to selecting PHA types tailored to specific user requirements and optimising their production through tailored engineering strategies. It emphasises the importance of end-user input, highlighting partnerships with

local companies like Senbis and H&P Moulding for filament and injection moulding applications, respectively.

Key learnings from the proof-of-concept pilot include the power of media optimisation in enhancing PHA production, the successful utilisation of textile-derived glucose, and the need for a clear product target market as well as quantitative product measurements to drive strain improvements.

TNO recommends that the initial enzymatic hydrolysis stage is scaled up adequately in order to overcome issues with insufficient volume prohibiting successful feeding into the reactor entrance. If significant scaling up could be achieved, then a larger gasification reactor could be available. In both cases, a focus on the downstream processes will be key in order to produce the desired end products of methane and/or methanol, which have the potential for direct use, as a chemical feedstock or potentially for biotechnology applications.

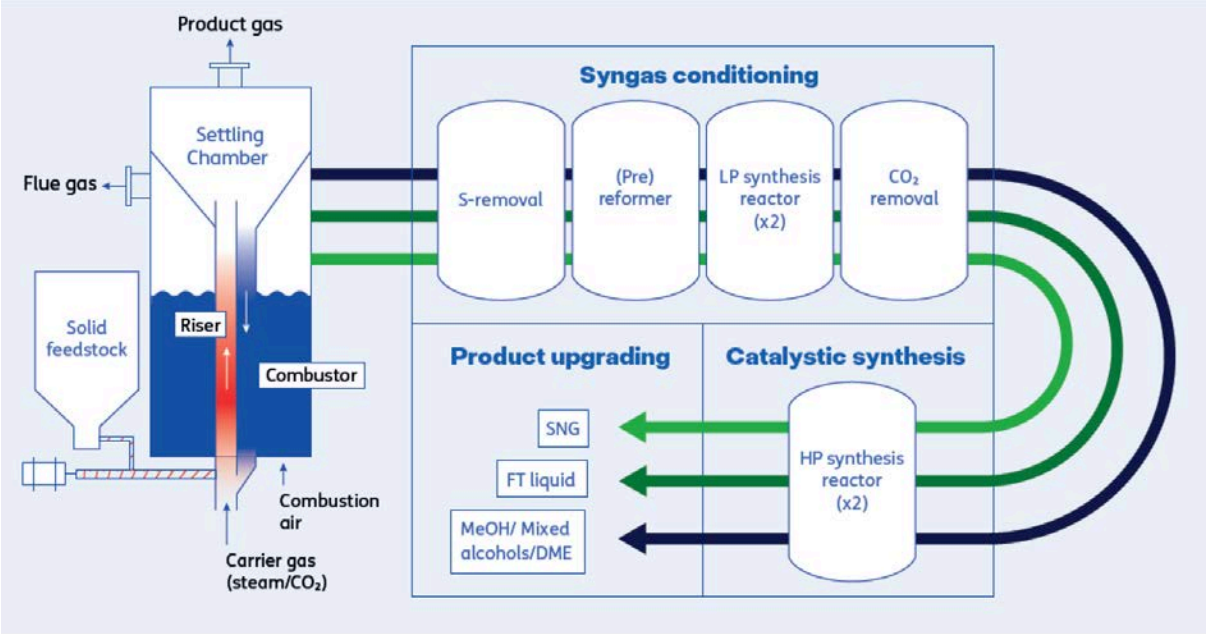


Figure 36. Schematic diagram showing the downstream processing steps that can be applied to upgrade (convert) syngas into a range of different end products.

D4T OUTCOMES ANALYSIS - CONCLUSION

Our initial proof-of-concept demonstrates the potential value of using enzymatic hydrolysis combined with thermochemical processing to yield commercially valuable end products from a waste stream that would otherwise be incinerated or landfilled. There is indication that high utilisation of glucose is possible, even without extensive filtration and purification. We need to continue to explore allowable contaminants in the case of no- or low-filtration and potential resulting downstream effects, whether positive or negative (e.g. equipment efficiency decline or improved bacterial growth curves). Likewise, we've found we can optimise the gasification of mixed waste toward feedstocks for the bioeconomy, but require more analytical testing to fully understand the impacts of variable feedstocks and processing conditions on end products and their ultimate commercial potential.

We propose the next stage of the pilot focus on a decomposition hierarchy, defining the highest and best use of materials in any given bioeconomic region. Moving toward a true bioeconomy model will require more than just proof of concept, modelling techno-economic assumptions and proving market desirability. We plan to closely partner with the technology partners who participated in the initial trial along with an expanded network of regional stakeholders—like the city of Rotterdam, Port of Rotterdam, and biotech experts such as Wageningen—to answer some of the remaining feasibility and scale questions.

5. NEXT STEPS

Building upon the results of this proof-of-concept pilot, the shared intention is to build a subsequent pilot taking on board all of the learnings from this stage whilst scaling up the processes and optimising towards desired end products. This would also involve conducting an economic analysis to move towards a business case for such a system, as well as measuring the associated social and environmental impacts.

In order for this project to be driven in the local region, the following project set-up is suggested:

- **Project lead**: overseeing all activities and connecting all parties involved, this would include project management
- **Technical lead:** validate approach from a technical perspective
- **Technology partners**: design and implement the pilot
- **Market consultant**: conduct economic analysis to form viable business case
- **Regional steering committee**: ensure that the pilot is being designed and developed in a way that would fit into the regional vision, market and infrastructure